

ANNUAL REVIEW OF PLANT PHYSIOLOGY

DANIEL I. ARNON, *Editor*
University of California

LEONARD MACHLIS, *Associate Editor*
University of California

VOLUME 3

1952

PUBLISHED BY
ANNUAL REVIEWS, INC.

ANNUAL REVIEWS, INC.
STANFORD, CALIFORNIA, U.S.A.

FOREIGN AGENCIES

H. K. Lewis & Company, Limited
136 Gower Street
London, W.C. 1

Maruzen Company, Limited
6, Tori-Nichome Nihonbashi
Tokyo

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA BY
GEORGE BANTA PUBLISHING COMPANY

531.1
A773
(1250)

131022

PREFACE

This volume of the *Review* is presented with the hope that it will deserve the wide acclaim given to the two preceding volumes. The growing influence of the young *Review* is attested by the frequency with which its name is already encountered in the scientific literature and the generous expression of approval and encouragement which the Editorial Committee has received from investigators, teachers, and advanced students of plant physiology and allied fields. Such encouragement can only mean that the reviews have been of high quality. We wish, therefore, to express once more our gratitude to the authors of the reviews to whose efforts this and the previous volumes owe their existence. We are also appreciative of the suggestions for both topics and authors which we have received from our colleagues, and we hope that we may count on their continued co-operation and interest.

The arrangement of literature citations continues to be a subject of discussion. In this matter the *Annual Review of Plant Physiology* has adopted the same principles as those which guide the other *Annual Reviews*. A full discussion of this subject will be found in the Preface to Volume 20 of the *Annual Review of Biochemistry*.

We wish to acknowledge the services of our editorial assistants and to thank our printers, the George Banta Publishing Company, for their fine co-operation.

D.R.G.	K.V.T.
P.K.	H.B.T.
M.W.P.	L.M.
D.I.A.	

111105

581.1
An78
(1952)

131962

TOPICS AND AUTHORS
ANNUAL REVIEW OF PLANT PHYSIOLOGY
VOLUME 4 (1953)

NITROGEN METABOLISM OF HIGHER PLANTS, *J. G. Wood*
INORGANIC NUTRITION WITH SPECIAL REFERENCE TO INTERACTION AMONG
ELEMENTS, *H. L. Crane and C. B. Shear*
PHOTOSYNTHESIS, *A. H. Brown and A. W. Frenkel*
RESPIRATION, *W. O. James*
ORGANIC ACIDS IN PLANT METABOLISM, *R. H. Burris*
HEMATIN COMPOUNDS IN PLANTS, *R. Hill and E. F. Hartree*
RELATION OF CHEMICAL STRUCTURE TO BIOLOGICAL ACTIVITY IN GROWTH
SUBSTANCE, *H. Veldstra*
PHYSIOLOGY OF FRUIT FORMATION, *J. P. Nitsch*
PHYSIOLOGY OF ROOT GROWTH, *H. Burström*
HERBICIDES, *A. S. Crafts*
TISSUE CULTURE, *P. F. Gautheret and G. C. Camus*
PLANT STEROLS, *W. Bergmann*
ALKALOIDS, *R. F. Dawson*
FLUORESCENT MATERIALS IN PLANTS, *R. H. Goodwin*
CERTAIN ASPECTS OF TERPENES OF INTEREST TO THE PLANT PHYSIOLOGIST,
J. Haagen-Smit
PHYSIOLOGY OF MYCORRHIZAL RELATIONS IN PLANTS, *E. Melin*
EFFECT OF TEMPERATURE ON PLANT GROWTH, *F. W. Went*
PHYSICAL PROPERTIES OF PROTOPLASM, *H. I. Virgin*

ERRATA

Volume 2

page 195, Ref. 55 and 56: *for Nieva read Sanchez*

CONTENTS

	PAGE
FACTORS AFFECTING AVAILABILITY OF INORGANIC NUTRIENTS IN SOILS WITH SPECIAL REFERENCE TO MICRO-NUTRIENT METALS, <i>G. W. Leeper</i>	1
GLYCOLYTIC ENZYMES IN HIGHER PLANTS, <i>P. K. Stumpf</i>	17
METABOLISM OF PHOSPHORYLATED COMPOUNDS IN PLANTS, <i>H. Albaum</i>	35
STUDIES OF THE PHYSIOLOGY, PHARMACOLOGY, AND BIOCHEMISTRY OF THE AUXINS, <i>J. Bonner and R. S. Bandurski</i>	59
AGRICULTURAL APPLICATION OF GROWTH REGULATORS AND THEIR PHYSIOLOGICAL BASIS, <i>J. van Overbeek</i>	87
TRANSPORT OF ORGANIC COMPOUNDS, <i>W. H. Arisz</i>	109
LEAF PROTEINS, <i>S. Wildman and A. Jagendorf</i>	131
PHYSIOLOGICAL ASPECTS OF LOW TEMPERATURE PRESERVATION OF PLANT PRODUCTS, <i>M. A. Joslyn and H. C. Diehl</i>	149
PHYSIOLOGY OF VIRUS DISEASES, <i>F. C. Bawden and N. W. Pirie</i>	171
MECHANISMS OF ION ABSORPTION BY ROOTS, <i>R. Overstreet and L. Jacobson</i>	189
PHYSIOLOGICAL BASES FOR ASSESSING THE NUTRITIONAL REQUIRE- MENTS OF PLANTS, <i>A. Ulrich</i>	207
PHOTOSYNTHESIS, <i>E. Rabinowitch</i>	229
PHYSIOLOGY OF FLOWERING, <i>A. Lang</i>	265
CARBOXYLATING ENZYMES IN PLANTS, <i>B. Vennesland and E. E. Conn</i>	307
TREE PHYSIOLOGY, <i>B. Huber</i>	333
INDEXES	347

Annual Reviews, Inc., and the Editors of its publications assume no responsibility for the statements expressed by the contributors to this *Review*.

FACTORS AFFECTING AVAILABILITY OF IN-ORGANIC NUTRIENTS IN SOILS WITH SPECIAL REFERENCE TO MICRO-NUTRIENT METALS¹

By G. W. LEEPER

*School of Agriculture, Melbourne University,
Melbourne, Australia*

INTRODUCTION

Soil chemists have centered their work for many years on two allied problems: first, to understand the mechanisms by which nutrients are supplied to plants; second, to estimate the ability of a particular soil to supply a crop with its nutrients. The two aims go hand in hand. One man may follow both in a single piece of work. Or perhaps one man may study equilibria in which phosphate ion is adsorbed on ferric compounds, while a colleague devises a practical test for estimating the amount of such adsorption by shaking a soil with a fluoride solution, which breaks the link between iron and phosphate. This review deals with the *principles* of availability. However, many of the articles reviewed are partly or wholly concerned with the *practical* question, "How much of this element can a given soil supply?" Innumerable methods of extraction exist for answering this practical question. While they do not constitute our subject, from time to time one of them will throw light on the underlying principles.

The major principles of availability have already been set out in the *Annual Reviews of Plant Physiology* (1, pp. 1-6; 2). In the course of two years there is little to add to these in general. It should be more useful to illustrate some principles in detail, and to give special attention to the micronutrient metals, since our ideas on these are more fluid than those on the well-known elements.

Every element passes through a cycle of changes between soil and solution, through higher plants and microorganisms, and back through the solution to the soil. The idea of such a cycle is most familiar with nitrogen, but it applies generally. We may divide the store of any element into two parts, according to whether or not each atom has entered the cycle since the soil began to form. Thus we may first consider the primary minerals of the coarser fraction of soil as a source of nutrients, and then consider what happens after the element has entered the cycle. The "coarser fraction" here means everything above 5 microns in diameter. Some might draw a different dividing line, but this does not affect the issue.

PRIMARY MINERALS

Different writers have given very different values to the primary miner-

¹ The survey of the literature pertaining to this review was concluded in September, 1951.

als as a source of nutrients. While no doubt most workers tacitly assume that they are valuable, many textbooks on soil disregard them. Some of the difference in attitude depends on whether the writer's interest is in temperate or equatorial soils. (The term "equatorial" gives a better contrast than "tropical" since so many tropical regions are dry for most of the year.)

In equatorial climates, primary minerals have long been looked on as a source of ready supply. Vageler, for example (3), suggests that equatorial soils are not worth cropping unless they contain this primary store. If they are to contain it, then the parent rock must be rich and the soil must be young. The very fact that under the warm rain the silicate crystals break up quickly enough to be useful to a crop means that they cannot keep on being useful for very many years. Van der Marel (4) has quoted Indonesian results in which the weathered fraction, the colloid, was almost useless, and practically all the crop's current supplies in different soils must have come respectively from bytownite (for calcium), hypersthene (for magnesium), and andesine (for potassium). At such a rate of decomposition, these crystals must disappear within a few centuries. Since the andesine, though available to crops, was little attacked even by a 2-hr. contact with concentrated hydrochloric acid, the direct microscopic study of the minerals of the sand fraction is evidently extremely valuable in equatorial soils.

In temperate climates, the primary minerals weather more slowly; how a drop of ten degrees in temperature can make such a vast difference is not easy to explain, but there it is. The crystals may weather too slowly to satisfy the current needs of crops, and may merely form a minimum reserve. Novak (5), reviewing the subject in 1938, calls the primary minerals the "iron rations" of the crop. Novak considers that this supply is negligible unless more than 10 per cent of the soil consists of primary minerals other than quartz, and considers between 10 and 20 per cent as "weak." Yet only an "immature" soil, which has not been much weathered, could contain such a large store as 20 per cent.

The term "primary mineral" includes every variety of crystal. Many workers have studied the rate at which different minerals weather and set free their useful elements. The silicate minerals of calcium, potassium, and magnesium have been specially investigated. Graham in particular (6) has used the plant to indicate the value both of individual minerals and of the coarse fractions isolated from various soil horizons. He suggests that the mechanism by which the mineral supplies the plant is through the attack by acid clay rather than by the carbonic acid of traditional teaching. At the same time, here, as throughout the problem, plants differ among themselves in their ability to use any one source. Lewis & Eisenmenger (7) suggest that the primary mineral is especially important for the more primitive forms of plants, which can absorb most of their needs of potassium from this source, while the more highly evolved plants cannot do so.

The minerals of the sand fractions have also been studied as a source of the micronutrients copper, cobalt, and zinc. These metals do not normally

make up more than a minute part of a primary crystal, but occur rather as "impurities." Thus their ions can replace magnesium in a crystal, but cannot replace the larger ions of calcium or sodium. The supply of ferromagnesian minerals in the sand fraction, as contrasted with feldspars, may therefore be used in a survey (8) to indicate the supply of these elements. This method was useful in parts of Western Australia where copper and cobalt are lacking in the parent material and hence in the soil.

However, such a geochemical approach, while useful in part, ignores all that happens after weathering. Taken alone, it would be less enlightened than the traditional extraction with boiling concentrated hydrochloric acid, which was at least supposed to distinguish between the inert unweathered crystals and the mobile weathered material. Many soils have long since lost by weathering any valuable primary minerals they may once have held, yet they still provide plants with all the nutrients they need. Many soils in temperate climates suffer from deficiencies in spite of containing primary minerals. Our main interest lies in what happens to the element after it has entered the cycle of solution-plant-animal-microbe; or perhaps simply solution-soil colloid or precipitate.

FROM AVAILABLE TO UNAVAILABLE

It is useful to approach availability from the negative side. What are the forms in which an element becomes unavailable? Applications of micro-nutrient salts have often failed to provide the needed supply, or have not had much residual effect. Many soils on which plants suffer from micro-nutrient deficiency contain a total of 1,000 times as much of the element as the crop needs. Yet apart from unweathered parent material, every atom of our element, whether from native or applied sources, has passed through the simplest ionic state at least once. What has happened to change it from that state? We will give our main attention throughout to the cations.

EXCHANGEABLE IONS

The first stage is to form the exchangeable ion attached to a negative colloid, whether organic or inorganic. This is generally considered to be the available state par excellence. However, taken alone, it often gives a poor correlation with the growth of plants. The amount of the ion accessible to plants depends on its proportion among the total adsorbed ions just as much as on its total amount. Stout & Overstreet (2) quote figures for the low availability of calcium when making less than 35 per cent of the total. Similarly, the availability of magnesium is lowered by introducing much potassium or sodium into the exchangeable ions. This effect is widespread on the sandy soils of Holland, where fertilizing with potassium is intensive (9). Marshall's work on activity, mentioned below, starts from this point.

The situation is different again when only minute amounts are present. If 1 p.p.m. of manganese is present as the exchangeable ion in a normal soil which contains, say 18 m.eq. of total exchangeable cations per 100 gm.,

then manganese makes up only 1/5,000 of the total cations. Copper may make up less than 1/100,000 of the total cations. Wiklander & Giesekeing (10) remark that the general belief is that these minute amounts are especially tightly held by the colloid and are therefore not easily available to plants; they then present experiments with radioactive tracers of potassium and strontium to show that the general belief is wrong, and that availability does not decrease further after the proportion of the wanted element has fallen below 1 per cent. The evidence which they found for potassium and strontium need not apply to the ions of the heavy metals, which have a different electronic structure. Stout & Overstreet (2), however, quote similar evidence for the heavy metals also where the proportion is below 0.1 per cent.

Steenbjerg has made detailed studies of the rate at which both copper (11) and manganese (12) are extracted from soils by electrolytes. He uses the empirical relation by which the proportion y/S of the ion extracted depends on the formula $y/S = x/(x + qS)$, where S is the total amount extractable, x is the volume of extracting solution, and q is a constant characteristic for each soil. Thus, where the ion is firmly held by the colloid, and is difficult to extract, qS is high. Some of the soils with low amounts of exchangeable copper or manganese give evidence of such low activity and firm binding.

Many soils have been analysed for exchangeable zinc and copper, and many more for exchangeable manganese. The outcome of all this is disappointing. In the first place the result of the analysis depends on the conditions. If the soil is allowed to dry before extraction it will liberate more manganese to the extracting salt (13) but less copper (11). Steenbjerg (11, p. 341) found that the "exchangeable" copper in one soil varied with the weather from week to week, passing from 46 gm. per acre in late June to 210 gm. in mid September, and back to 57 gm. in November. He put down the autumn rise to greater moisture, but could not explain the winter fall. The salt used for extraction is also far more critical than it is when the better-known cations are analysed. Calcium and magnesium salts may extract 10 times as much manganese as do the corresponding salts of sodium and ammonium. This effect should be expected since the heavy-metal ions are so tightly adsorbed that a univalent ion cannot easily remove them. On the other hand, Bondorff (14) has quoted examples of the opposite order, in which he believes the univalent ion allows organic colloid to disperse and so carry manganese into the filtrate. The anion of the replacing salt is also important, as is discussed later. Even when all precautions are taken to make analyses comparable, the correlation with the health of plants is still not good enough; there is still too much overlap in one and the same investigation, both for copper and for manganese, between the values for normal and deficient soils. This could be due to two causes: first, that the exchangeable form of these less mobile ions is not as readily available as is often assumed; second, that non-exchangeable sources, if large enough in amount, may be adequate. Thus, in considering our sequence of compounds of decreasing availability, we must remember that a greater bulk may make up for lower quality.

For exchangeable manganese, many workers have found that about 1 p.p.m. is a dividing line between fertile and deficient soils. The most recent such observation is from Brittany [Coppenet & Voix (15)]. However, these authors, who leach with ammonium acetate, point out that their method is highly conventional and their figures apply only to their own conditions. For example, they extract 50 per cent more manganese by shaking for 3 hr. than by their standard method of leaching for the same time. Other French workers (16), as well as the reviewer (17), have shown that the overlap among different investigators is large, and Jones & Leeper (18) have found that among four soils with various treatments aimed at overcoming deficiency, the exchangeable manganese extracted by shaking with calcium nitrate was of the same low order ($\frac{1}{2}$ to 1 p.p.m.), whether the treatment succeeded or not.

Steenbjerg & Boken (19), after collecting thousands of results for soils in Jutland with ample or deficient copper, conclude that the best correlation is given by extracts with dilute acid adjusted to give a final pH of 2. Such extracts must contain more than strictly exchangeable copper; so this too means withdrawing from the search for exchangeable forms.

Other adsorbents.—Exchangeable cations are usually thought of as being adsorbed on the colloidal particles of the clay minerals or of the organic fraction. In a calcareous soil, however, calcium carbonate may be an even more powerful adsorbent. Calcium carbonate has long been used as an adsorbing material for removing minute amounts of heavy metals from culture solutions. The equilibrium concentration of these metals is thus brought far below the level given by the solubility product for metallic hydroxide or carbonate. Boisshot *et al.* (20) believe that this effect applies to manganese in the soil (by implication, to other heavy metals also). They show that manganese, adsorbed from solution onto calcium carbonate, may be released again by neutral ammonium acetate.

Aluminum oxide as prepared for chromatography is an even more efficient scavenger for culture work with the heavy metals (20a).

COMPLEX FORMATION

Analyses for the "exchangeable" form of the heavy metals give confusing results, and applications of their salts to soil have often failed to cure a deficiency. It has been too readily assumed that their ions are adsorbed in the same way as the "rare gas" ions of calcium and magnesium, that is, by electrostatic attraction. They can be more tightly adsorbed than this, however, probably because the atoms easily form covalent links and chelate compounds. Their adsorption need not be reversible. Covalent linkage could explain the slowness with which manganese in particular is exchangeable, a fact which many workers have noted. It could also explain how the heavy metals become much less mobile as the soil pH changes from acid to neutral. This last observation has often been made. Peech (21) makes it the basis of advice that the deep sands of Florida should be kept at a soil pH of

6, at which value the heavy metals are just mobile enough to be available to plants, but not enough to be washed out by rain. This lower mobility has been put down to the formation of hydrolysed or other complex ions such as $(\text{CuOH})^+$ and $(\text{ZnCl})^+$. No doubt such ions are present (22), but it is difficult to see why they should be more tightly adsorbed than the simple cupric and zinc ions. A change in acidity, on the other hand, could greatly affect a linkage which had some covalent character.

The main suggestions of insoluble complexes of the heavy metals come from work on soils high in organic matter. Instances of extreme deficiencies have been reported from these soils. The insoluble complexes may be thought of as analogous to such precipitates as those formed by various metals with hydroxyquinoline. In soils, however, the chelating compound is of colloidal size, with the oxygen, nitrogen, and sulfur atoms of the colloid molecule all able to take part in the binding. A metal so held will not be displaced by ammonium or calcium salts in the determination of exchangeable cations, but will stay in the insoluble state until the organic holding group weathers away under microbial attack. Many workers on the chemistry of copper and manganese in soil have stated or implied that CuX and MnX (where X stands for a whole colloidal particle) represent the unavailable forms into which the metal may pass within a few weeks after being added to the soil. No doubt such compounds exist; the question is whether surface complexing is to be regarded as the main reason for low availability.

The "X hypothesis" is more plausible for copper than for manganese. Lees (23) has isolated the humic acid fraction from peat and found that it retained 15 out of a total of 20 microequivalents of copper present when in equilibrium with 11 ml. of .5 M calcium nitrate. He takes this as evidence of complexing, but his figures do not show anything more than the well-known strong competing power of copper among the exchangeable cations. In fact, there is no clear dividing line between a firmly held exchangeable cation and a complexed, chelated atom.

Mulder (24) has recorded how copper deficiency disease was once attributed to an organic toxin, "gliedin," which could even be extracted. The copper salt was supposed to be an antitoxin. However, this gliedin has never been confirmed and the X hypothesis of today is more attractive: copper is added to saturate the X and not to destroy the gliedin. Further, the fact that deficient soils are improved or cured by autoclaving could be explained if X is destroyed. Even so, the X hypothesis will remain speculative until someone can isolate the X from a deficient soil, add it to a normal soil, and so cause a micronutrient deficiency. It should be remembered here that many of the deficient soils are not high in organic matter. A widespread kind of copper-deficient soil which Steenbjerg studied was a sand with only 4 per cent of organic matter (11, 19).

Some results which bear on this hypothesis are derived from the use of sodium or potassium pyrophosphate solutions (preferably potassium, since its salt is the more soluble). Pyrophosphate dissolves from soils both organic colloid and heavy metals. A group of workers at Rothamsted Experimental

Station (25) first observed this reaction when treating soil with a compound labeled "anhydrous Na_2HPO_4 ." The use of this commercial salt must often have led to queer results, since it is difficult to remove water of crystallization from the orthophosphate without producing pyrophosphate, which unlike orthophosphate is a vigorous complex-forming ion.

Pyrophosphate acts in two ways. It combines with calcium and with heavy-metal ions. By the first effect, the whole organic colloid peptizes, carrying with it heavy metals which were bound as CuX , MnX , etc. By the second effect, which these authors rely on, metal-pyrophosphate crystalloids are formed both from metallic oxides and from the organic complexes, since pyrophosphate competes strongly with other complex-formers such as X . One cannot say without further study from which of these sources a metal has been extracted.

Heintze & Mann in their latest paper (26) give evidence for the existence of substantial amounts of bivalent manganese as a complex in neutral soils high in organic matter. (They consider that plants can obtain little or none of this manganese.) They use alkaline pyrophosphate at pH 9.4, which can form the stable ion $\text{MnP}_2\text{O}_7^{-2}$ and thus compete with the rival X . The reaction appears to be:



going from left to right at pH 9.4 and from right to left at pH 7.0, pyrophosphoric acid being only half dissociated at the lower pH. Their argument is often difficult to follow, but the above equation written by the reviewer seems a fair summary. Heintze & Mann express some doubt as to the origin of the bivalent manganese which they extract. It may also arise by reduction of MnO_2 or some other oxidized form of manganese, X now appearing as a reductant



soil organic matter develops new reducing groups in an alkaline solution [Mattson *et al.*, (27, 44)], and so comes closer to its high reducing power under acid conditions. This possibility of reduction of higher oxides often makes laboratory results with manganese difficult to interpret. The slow release during leaching could be due to the slow breaking of a bond, or to slow reduction; we do not know which.

Eriksson (28), in reporting studies of the complex constant of $\text{CuP}_2\text{O}_7^{-2}$, suggests that pyrophosphate should be a good extractant for available copper in soils, since it is a stronger complex former than any likely organic groups containing oxygen, though its competitive powers against nitrogen and sulfur groups are unknown.

PRECIPITATES WITH SIMPLE ANIONS

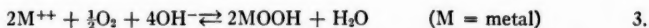
Instead of being adsorbed, loosely or tightly, on the major negative colloids of the soil, a cation may form a precipitate with a simple anion—hydroxide, carbonate, phosphate or silicate. Zinc, manganese, copper, and

cobalt are generally found to become less available to plants with increasing alkalinity. This subject can be approached as was done once by Britton, who measured the pH at which a precipitate of "hydroxide" or basic salt began to form on titrating a chloride or sulphate solution of the cation with alkali (29). Britton's values are sometimes quoted in discussions about soil, but they do not help us much. Britton used .02 *M* solutions, whereas a nutrient solution in which the copper concentration is only 10^{-6} *M* can grow a healthy crop of oats (30). If we insert the appropriate figures for the solubility products of the hydroxides, say at pH 7, we still cannot account for the failure of soils to provide enough of these elements in solution. Neither does it help to regard the carbonate as the precipitating ion; the solubility products are again too high.

OXIDATION

The only two elements to consider here are iron and manganese, though cobalt may also find a place eventually. The bivalent ions of iron and manganese may exist in solution or in the adsorbed form; the availability of such forms at this lower valence has already been discussed. These bivalent ions may be oxidized to give products which are insoluble at the common range of soil pH; namely, oxides or hydrous oxides, in which the valence of the metal is 3 for iron, and 3 or 4 for manganese. All these oxides will here be called "higher oxides."

The major changes from the reduced to the oxidized state have long been recognized; alkalinity and aeration favour oxidation, acidity and water-logging favour reduction.



There has been much discussion as to how far this oxidation is biological. With manganese, there is no doubt that the microflora cause far more rapid oxidation than would happen *in vitro* with manganous sulphate at the same pH, but they appear to act indirectly by excreting metabolites which catalyze autooxidation, rather than by direct metabolic action on the metal [Bromfield & Skerman (31)]. With iron, the oxidation could easily be nonbiological, since the redox potential of the system $Fe^{++}:Fe(OH)_3$ is 0.5 v. lower at neutrality than that of $Mn^{++}:MnO_2$; but even here, Gleen has found that some of the oxidation is microbial (32). Whatever may be the mechanism of these various oxidations, they appear to go vigorously in all neutral and alkaline soils that are not waterlogged. Only by forming an exceptionally tight complex can the metal avoid oxidation.

At the same time, the higher oxides can be reduced either by organisms or by the more reactive fraction of the soil organic matter. The reducing properties of organic fractions have been little studied to date, but should reveal some interesting distinctions among soils. By whatever mechanism it is formed, however, the amount of reduced ion at equilibrium at neutrality is normally less than one-hundredth of that of the higher oxide (17). A possible exception has already been mentioned (26).

The nature of the higher oxide is important. While iron has a higher valence only of 3, manganese has the two possible valences of 3 or 4. The valence of 4 is unambiguous in MnO_2 , but that of 3 is harder to establish. Mn_2O_3 can as easily be $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}\text{O}_3$ as $\text{Mn}^{\text{III}}\text{Mn}^{\text{III}}\text{O}_3$. Even if we extract with a special reagent for Mn^{III} , we still cannot be certain that this intermediate valence was that of the compound tested, since the reversible action



has only a low energy content near the neutral point, and can be sent from left to right or right to left at will by adjusting the environment. Thus Heintze & Mann (26) have found that pyrophosphate favours the forward action at pH 7 and the reverse action at pH 9.4, because these pH's favour the stability of complexes with tri- and bivalent manganese respectively. On that account these authors are noncommittal about establishing the valence of the oxidized manganese in the soil. Fyfe (33) seems to have found an unambiguous proof for a valence of 4 in the oxides hausmannite (Mn_3O_4) and bixbyite (Mn_2O_3). His reagent is a mixture of acetylacetone and hydrochloric acid. Trivalent manganese forms a chelated complex and does not react further, while quadrivalent manganese forms the complex and liberates chlorine at the same time. This problem has also been reviewed by Wadsley & Walkley (34), who conclude that no reliable method has been developed whereby the valency state of the metal in a particular manganese oxide may be found.

Whatever the formula of the higher oxides, most workers have regarded the oxidation of iron and manganese as another form of degradation from an available to an unavailable form. This common opinion is plausible in that the bivalent ion can come into solution, while the higher oxide is insoluble. The most successful application of this view in the field has been the cure of manganese deficiency in a soil by adding acid peat treated with manganous sulphate [Delap (35)]; the bivalent ion adsorbed on the peat was just mobile enough to be used by plants, but not mobile enough to enter into the normal cycle of oxidation. A similar and more surprising success has been claimed for peat plus ferrous sulphate (36). However, it is a mistake to confine attention to forms that are generally so poorly represented in the range of soil pH from 6 to 8.

Manganese.—The reviewer's work from 1934 onwards (17, 37) has avoided the confused field of "exchangeable" manganese and has emphasized rather the supply of higher oxide which is rapidly dissolved by mild reducing agents such as quinol. This supply is commonly high in those alkaline soils from which plants obtain ample manganese though the exchangeable form may be low. The higher oxides of manganese should therefore be thought of as a direct source of this element for plants. A good supply in an acid soil (perhaps 50 p.p.m. is enough) is then a sign that liming is not likely to cause deficiency disease. Heintze, while not generally in agreement with the reviewer, assents to this last statement (38, p. 229).

Bolas & Portsmouth (39) find that simply a high concentration of carbon

dioxide causes enough higher oxide to dissolve to protect plants against manganese deficiency disease. (The acidity allows easier reduction by the organic matter.) One might think of the active oxides simply as a reservoir from which an occasional reducing molecule might liberate a manganous ion to replenish the exchangeable supply. If so, it is hard to see why so many normal soils should be so low in the exchangeable ion.

Jones & Leeper (40) have found in pot tests that some synthetic higher oxides when added to naturally deficient soils cause healthy growth of oats. The two most successful oxides had the crystal structures of pyrolusite and of the curiously named "manganous manganite," both of a composition close to MnO_2 . Only the most finely divided oxides, with particles in the colloid range, were successful. Hausmannite (Mn_3O_4), however fine, was a failure but manganite ($MnOOH$) came close to pyrolusite.

Iron.—Iron is peculiar among the micronutrients. A chlorosis which could be cured by iron sprays was long ago recognized among sensitive plants growing on calcareous soils. Yet the total amount of iron in such chlorotic plants is usually high. Bennett's work (41) certainly shows a low iron in chlorotic plants, after washing the leaves with dilute acid; but the weight of evidence is on the other side, as stated. Either the iron enters the plant in a less useful form than normal, or something goes wrong with the reactions of iron inside plants growing on certain soils. The latter seems more likely. It presents a problem to plant physiologists which so far has hardly been touched. Since this review, however, is concerned with the absorption of the element by the root, and not with its subsequent fate, we must consider in what form iron can be absorbed.

Kliman (42) has presented evidence that the reduced bivalent ion is the form absorbed by plants. This ion must have only a short life in most soils which have a pH between 6 and 8. At the same time solid magnetite (Fe_3O_4) is an adequate source of iron in quartz sand cultures [Chapman (43)]. We may picture then a mechanism of the root for local reduction and absorption analogous to "contact exchange" (2, p. 323) for both manganese and iron. Such a mechanism should be expected to vary greatly among plants, which themselves vary in their power to use a given source in a soil [see Mattson *et al.* (44)]. The mechanism also would depend on the crystalline type of the oxide and the nature and extent of its surface. It may turn out that a reagent like calcium thioglycolate [Paddick (45)], acting for a short time, may extract the useful sources of iron oxide, as quinol does with manganese oxide.

On the other hand, a mechanism for reducing iron to a ferrous ion may not be involved. It may be better to consider another possibility, as set out in the next section.

SOLUBLE COMPLEXES

We have seen that pyrophosphate can form soluble complexes which are stable even in the presence of strongly adsorbing solids. Soluble complexes of this nature may be important in the soil. It may be that heavy metals are

absorbed as complex anions to an extent that is not at present recognized.

Pyrophosphate forms stable complexes with the ferric, manganous, manganic, and cupric ions, among others. Mattson *et al.* (44) have suggested that manganese deficiency might be cured by adding pyrophosphate to the soil and so dissolving Mn_2O_3 . However, no success has yet been achieved and perhaps none should be expected from this procedure, since pyrophosphate is easily hydrolysed in soil, and besides, the amount of manganese extracted from soil with neutral pyrophosphate bears no relation to the health of plants. The only success reported with polyphosphates to date seems to be that of a metaphosphate solution together with ferric tartrate in providing apple trees with ferric iron [Edgerton (46)]. It may be, of course, that synthetic media have often contained meta- and pyrophosphates unintentionally produced by drying an orthophosphate too strongly.

The best-known soluble complexes in plant culture are ferric tartrate and ferric citrate, which are often used as the plant's only source of iron. The iron in these is anionic, being chelated with hydroxyl groups. Perhaps some plants in the field get much of their iron in these and analogous forms; if so, the flora of the rhizosphere must play a major part in producing the chelating acids, and this must vary from plant to plant. Iron in neutral soils cannot be soluble except in such complexes; the free ferrous ion will be oxidized and the free ferric will be hydrolysed. The other heavy metals need not be chelated in order to be sufficiently soluble, but soluble complexes may help the total intake. Trivalent manganese also forms complexes with the hydroxyacids, but these are less stable than the ferric complexes, and less likely to be a major source of the element.

A difficulty in analytical work may be mentioned here. Acetate ion also forms stable complexes with the heavy metals, even though it cannot compare with citrate. Copper, for example, in a normal solution of ammonium acetate, is reported to contain less than one-hundredth of its atoms as the simple cupric ion, the rest being more or less associated with acetate [Pedersen (47)]. [It should be pointed out that the concentration of cuprammonium ion, which has been attributed to such solutions (26, p. 93), must be minute.] The acetate ion in extracting solutions has its own solvent role, to an extent that has not been realised. This statement must apply to manganese (40), to cobalt [Young (48)], and to iron, as well as to copper.

A surprising suggestion has come in papers by Bastisse and colleagues (49), that iron may be absorbed by way of a negative ferrisilicate sol. Unlike the usual positively charged ferric sols, the ferrisilicate has the same charge as the other soil colloids; once formed, it can remain mobile. Bastisse has claimed success with a dried and redispersible ferrisilicate (50).

AGING AND RECRYSTALLIZING

Aging is the culmination of unavailability. It is best introduced by considering the importance of knowing how much surface of the wanted element is exposed.

Most estimates of the supply of an element available to plants are expressed in terms of weight, so many parts per million of soil, or so many pounds per acre to a depth of 6 in. This difference between expressing in terms of weight or of volume is important when dealing with peat soils. But when we record the element as so many grams or pounds per acre, we imply that the soil is uniform, which it is not. This does not matter for a soluble ion (such as nitrate) or for a mobile adsorbed ion (such as potassium), but the less mobile the element, the more serious is the mistake. Evidently we should think in different terms of a coarse and a fine application of an insoluble fertilizer. From this it is a short step to calculate not the weight, but the area of the source of supply in contact with the root. When micro-nutrients are added to soil as insoluble compounds, fineness of division may make the difference between success and failure. Steenbjerg's work with copper minerals (51) provides an example. Using different sizes of grains of sulphides, oxide, and carbonate on deficient soils in pots he finds that the good effect on the plant depends on the surface area of the mineral, not on its total weight. Jones & Leeper's work on manganese oxides (40) illustrates the same point. Pyrolusite is useless when applied to deficient soils as crystals several microns thick, but is successful on the same soils as a highly dispersed powder prepared in the wet way. The detailed structure of the surface must also be important for the availability of insoluble sources like the higher oxides of iron and manganese. Catalytic activity of oxides, the performance of MnO_2 in Leclanche cells, the use of iron oxides for removing hydrogen sulphide from coal gas, all lead to the same idea.

A precipitate changes from the moment of its formation; its ions rearrange themselves to give a more ordered, more compact, and less reactive product. This change is known as "aging," and is familiar in analytical work. Aluminium hydroxide, for example, dissolves easily in dilute acid when freshly formed but not after standing a few hours. Now the residual amount of an element applied as fertilizer for a previous crop may not have much effect on the succeeding crop some months later. This cannot be due simply to precipitation, which must be immediate when salts of heavy metals are added to all but the sandiest soils. It can rather be thought of as aging, which involves both a smaller and a different kind of surface. The old term "reversion," which has especially been used for the loss in activity of added phosphate, should be interpreted to mean not only an immediate precipitation, but a further recrystallization. Some recrystallized products of phosphate in soils have recently been studied (52). A similar recrystallizing is suggested for the poor residual effects of some manganese oxides [Jones & Leeper (18)]. In fact, our problems always remind us that the precipitations that we study are not reversible.

A type of recrystallizing which has been much studied is the entry of potassium ions into the lattice of some kinds of clay (2). A similar entry has been suggested for zinc (53), which might take the place of magnesium in a lattice. Elements which thus become incorporated in a crystal may be lost for years before being released again to the cycle.

This is a convenient point at which to refer to the work of Trocmé, Barbier & Chabannes (16), since they too emphasize the importance of surface exposed. They studied the alluvial sands of the Seine, which have been irrigated for over 50 years with liquid sewage from Paris, and where manganese deficiency of vegetable crops has recently become acute on areas receiving the heaviest irrigation. (As much as 13 ft. has been applied.) This heavy irrigation has removed half the soil's original store of manganese (the active half!) and has raised the organic matter to 9 per cent, as compared with 4 per cent in the healthy soil. Added manganous sulphate or manganous carbonate, even at the rate of 160 lb. manganese per acre, had no effect. Trocmé *et al.* do not believe that mere oxidation is to blame, since manganese added to the nearby normal soil is just as completely oxidized. They blame rather the organic colloid, but not in the usual sense of forming an X-complex with bivalent manganese. On the contrary, they stored mixtures of manganous sulphate and soil with varying amounts of organic colloid and showed that the addition of organic colloid actually increased the amount of exchangeable manganese. However, the organic colloid greatly decreased the manganese extracted from higher oxides by the weak reducing agent, quinol. The suggestion is that the colloid coats the surface of the particles of higher oxide and so holds both roots and laboratory reagents away from this store. Reduction of surface in this way is just as effective as any precipitation or crystallization.

ACTIVITY MEASUREMENTS

The foregoing account refers to the ceaseless search for reagents which extract the "active" fraction of an element, especially that fraction from which the plants can draw their supply for the current season. In recent years the idea has arisen that instead of extracting the soil we might directly estimate the most active fraction electrometrically. The greatest amount of work on these lines is that of Marshall's school, the findings of which were summarized in 1950 (54). Marshall has used treated silicate membranes dipped into pastes of clay minerals to estimate the active concentration of the four main cations (sodium, potassium, calcium, magnesium) and has shown to a surprising degree how the activity of calcium and magnesium is lowered by the presence of much sodium or potassium. This work fits in with two observations; (a) that magnesium-deficiency symptoms in oats may be induced in some soils by adding any of the other three cations, especially potassium; and (b) that plants growing on some alkaline soils, with magnesium and sodium dominating among the exchangeable ions, are low in calcium even though the soil is rich in calcium carbonate.

Russell & Cox (55) also remind the reader that the amount of any exchangeable cation bears no simple relation to its activity. They go on to report ambitious experiments, which are as yet preliminary. They hope to be able to measure activities of many ions by an indirect electrometric method; for example, they measure the activity of aluminum by the potential of a zinc rod in a paste of zinc ferrocyanide. When such methods are developed,

they may still fall short of the object of measuring what is available. We still have no idea how far plants absorb the heavy metals in the chelated form as anions. Russell & Cox believe that iron is so absorbed and cannot therefore be studied by their method.

COMPETITION BY MICROORGANISMS

The microbial flora bring a degree of uncertainty into all attempts to estimate the supply of an element by extracting a soil in the laboratory. They may cause the supply to be richer or poorer than appears from the analysis. Locally high production of carbonic acid or of chelating hydroxy acids like citric may dissolve sources of an element which the unaided root cannot reach [see, for example, Gerretsen's work with phosphorus (56)]. On the other hand, the microflora use the same elements as the higher plants, and may reduce an already small reserve below the minimum needed by plants. The two effects may appear in the same soil.

The best-known example of damage by the normal microflora is seen when a carbohydrate is added to soil and the microflora, using this as a source of energy, multiply and absorb the scanty supply of inorganic nitrogen. A similar effect is noted for phosphorus [Kaila (57)]. These effects are temporary, but may be decisive for any one crop. After a period of bare fallow the microbial population has fallen and the nutrients in the dead cells become available again. It is well known that nitrate ion thus becomes plentiful after a period of shortage. Millikan (58) reports that a shortage of zinc is worse on some soils plowed from cereal stubble than after a period of fallow.

If we make the rough approximation that the amount of an element in microbial cells equals that in a normal crop covering the whole area, we can see that microbial competition is only important when the supply of the element is chronically low, or when a fresh source of energy has recently been added. Where the supply is low, however, competition must be serious, since the absorbing surface of microbial cells is many times that of the root. Not much work has been carried out to estimate how much plants may lose in this way. The main method of study is by sterilizing the soil, whether by heat or by germicides. Many experiments have shown that deficiencies of copper, manganese, and zinc have been cured by either treatment. Heating, usually by autoclaving for 2 hr. or more, is very drastic chemically, and must destroy some of the groups to which the metals are combined. A germicide is theoretically preferable, but whether heat or a chemically unreactive germicide like formaldehyde is used, the bad effect of competition may be proved by inoculating the treated soil with the untreated, and bringing back the symptoms of deficiency. Ark (59) reports such an experiment for zinc, and Millikan's results (58) give some support. Gerretsen's similar experiment with manganese (60) could be quoted here also, although he regards the microbial flora in deficient soils as doing harm quite apart from competition. Jones & Tio (61) report a similar experiment for iron. These last workers at-

tribute "frenching" of tobacco to iron deficiency, and since the trouble occurs at a temperature of 35°C. but not at 21°, they believe that the competing microorganism is stimulated more than the plant by the higher temperature. At the same time, a higher temperature can also work in favour of the plant. Many examples are recorded where a plant has suffered from a micronutrient deficiency in its early growth and has recovered later. This could be due to solution by new compounds produced in the rhizosphere, or perhaps to a non-biological higher solubility at a higher temperature.

Swaby in unpublished experiments has found the two opposing effects of microorganisms—solution and competition—on the same plant, subterranean clover, growing in sand cultures. When iron and zinc were omitted, the plants grew much better when inoculated with soil microflora and a source of energy, while in the same experiment the microflora made the deficiencies worse, in the case of manganese and molybdenum.

LITERATURE CITED

1. Mulder, E. G., *Ann. Rev. Plant Physiol.*, **1**, 1-24 (1950)
2. Stout, P. R., and Overstreet, R., *Ann. Rev. Plant Physiol.*, **1**, 305-42 (1950)
3. Vageler, P. *An Introduction to Tropical Soils* (Greene, H., Tr., The Macmillan Co., 240 pp., 1933)
4. Van der Marel, H. W., *Soil Sci.*, **64**, 445-51 (1947)
5. Novak, V., *Trans. 1st Commission Inter. Soc. Soil Sci.*, **A**, 30-37 (1938)
6. Graham, E. R., *Proc. Soil Sci. Soc. Am.*, **6**, 259-62 (1942)
7. Lewis, C. C. and Eisenmenger, W. S., *Soil Sci.*, **65**, 495-500 (1948)
8. Teakle, L. J. H., Burvill, G. H., and Carroll, D., *Australian J. Sci.*, **4**, 40-44 (1941)
9. Mulder, D., *Trans. 4th Intern. Congr. Soil Sci.*, **II**, 141-44 (1950)
10. Wiklander, L., and Giesekeing, J. E., *Soil Sci.*, **66**, 377-384 (1948)
11. Steenbjerg, F., *Tids. Planteavl*, **45**, 259-368 (1940)
12. Steenbjerg, F., *Tids. Planteavl*, **39**, 401-41 (1933); **40**, 337-71 (1934)
13. Sherman, G. D., and Harmer, P. H., *Proc. Soil Sci. Soc. Am.*, **7**, 398-405 (1943)
14. Bondorff, K. A., *Tids. Planteavl*, **53**, 443-48 (1950)
15. Coppenet, M., and Voix, S., *Ann. inst. natl. recherche agron., Ann. agron.*, [A]1, 119-23 (1950)
16. Trocmé, S., Barbier, G., and Chabannes, J., *Ann. inst. natl. recherche agron., Ann. agron.*, [A]1, 663-85 (1950)
17. Leeper, G. W., *Soil Sci.*, **63**, 79-94 (1947)
18. Jones, L. H. P., and Leeper, G. W., *Plant and Soil*, **3**, 154-59 (1951)
19. Steenbjerg, F., and Boken, E., *Plant and Soil*, **2**, 195-221 (1950)
20. Boisshot, P., Durroux, M., and Sylvestre, G., *Ann. inst. natl. recherche agron., Ann. agron.*, [A]1, 307-15 (1950)
- 20a. Swaby, R. J. (Personal communication)
21. Peech, M., *Soil Sci.*, **51**, 473-86 (1941)
22. Menzel, R. G., and Jackson, M. L., *Trans. 4th Intern. Congr. Soil Sci.*, **I**, 125-28 (1950)
23. Lees, H., *Biochem. J.*, **46**, 450-51 (1950)
24. Mulder, E. G., *Over de betekenis van koper voor de groei van planten en micro-organismen* (Doctoral thesis, Agricultural Univ., Wageningen, Holland, 1938)

25. Bremner, M., Mann, P. J. G., Heintze, S. G., and Lees, H., *Nature*, **158**, 790-91 (1946)
26. Heintze, S. G., and Mann, P. J. G., *J. Agr. Sci.*, **39**, 80-95 (1949)
27. Mattson, S., Eriksson, E., and Vahtras, K., *Kgl. Lantbruks-Högskol. Ann.*, **15**, 291-307 (1948)
28. Eriksson, E., *Kgl. Lantbruks-Högskol. Ann.*, **16**, 72-83 (1949)
29. Britton, H. T. S., *Ann. Rept. Chem. Soc.*, **40**, 43-59 (1943)
30. Piper, C. S., *J. Agr. Sci.*, **32**, 143-78 (1942)
31. Bromfield, S. M., and Skerman, V. B. D., *Soil Sci.*, **69**, 337-48 (1950)
32. Gleen, H., *Nature*, **166**, 781-72 (1950)
33. Fyfe, W. S., *Anal. Chem.*, **23**, 174-75 (1951)
34. Wadsley, A. D., and Walkley A., *Rev. Pure and Applied Chem.*, **1**, 203-13 (1951)
35. Delap, A. V., *Ann. Rept. East Malling Research Sta. Kent.*, **37**, 116-17 (1949)
36. Pizer, N. H., and Downes, W. F., *Agriculture (Engl.)*, **52**, 120-22 (1945)
37. Leeper, G. W., *Nature*, **134**, 972-73 (1934)
38. Heintze, S. G., *J. Agr. Sci.*, **36**, 227-37 (1946)
39. Bolas, B. D., and Portsmouth, G. B., *Nature*, **162**, 737 (1948)
40. Jones, L. H. P., and Leeper, G. W., *Plant and Soil*, **3**, 141-53 (1951)
41. Bennett, H. P., *Soil Sci.*, **60**, 91-105 (1945)
42. Kliman, S., *Proc. Soil Sci. Soc. Am.*, **2**, 385-392 (1938)
43. Chapman, H. D., *Soil Sci.*, **48**, 309-14 (1939)
44. Mattson, S., *Kgl. Lantbruks-Högskol. Ann.*, **11**, 135-44 (1943)
45. Paddick, M. E., *Proc. Soil Sci. Soc. Am.*, **13**, 197-99 (1949)
46. Edgerton, L. J., *Proc. Am. Soc. Hort. Sci.*, **41**, 237-39 (1942)
47. Pedersen, K. J., *Kgl. Danske Videnskab. Selskab., Mat-Fys. Medd.*, **22**(12) (1945)
48. Young, R. A., *Proc. Soil Sci. Soc. Am.*, **13**, 122-26 (1949)
49. Demolon, A., and Bastisse, E. M., *Ann. agron.*, **14**, 265-96 (1944)
50. Bastisse, E. M., *Ann. agron.*, **16**, 434-46 (1946)
51. Steenbjerg, F., *Tids. Planteavl*, **47**, 557-99 (1943)
52. Haseman, J. F., Brown, E. H., and Whitt, C. D., *Soil Sci.*, **70**, 257-71 (1950)
53. Elgabaly, M. M., *Soil Sci.*, **69**, 167-73 (1950)
54. Marshall, C. E., *Trans. 4th Intern. Congr. Soil Sci.*, **I**, 71-82 (1950)
55. Russell, E. W., and Cox, G. A., *Trans. 4th Intern. Congr. Soil Sci.*, **I**, 138-41 (1950)
56. Gerretsen, F. C., *Plant and Soil*, **1**, 51-81 (1949)
57. Kaila, A., *Soil Sci.*, **68**, 279-89 (1949)
58. Millikan, C. R., *Proc. Roy. Soc. Victoria*, **54**, 145-95 (1942)
59. Ark, P. A., *Proc. Am. Soc. Hort. Sci.*, **34**, 216-21 (1937)
60. Gerretsen, F. C., *Ann. Botany*, **1**, 207-30 (1937)
61. Jones, L. H., and Tio, M.A., *Plant Physiol.*, **23**, 576-94 (1948)

GLYCOLYTIC ENZYMES IN HIGHER PLANTS¹

By P. K. STUMPF

Division of Plant Biochemistry, University of California, Berkeley, California

INTRODUCTION

This review will be restricted to a critical evaluation of the investigations of the enzyme systems in higher plants which participate in the phosphorylytic breakdown of carbohydrates to fermentation products. Much of the literature dealing with related topics has been recently discussed by Hassid & Putman (1), and by Goddard & Meeuse (2). To avoid duplication, some of the work on phosphorylated compounds found in plant tissues will be omitted since this material is treated by Albaum in this volume.

Although the important discoveries of Meyerhof, Cori, and others greatly stimulated research on the glycolytic systems of animal tissues and yeast, work of this nature with tissues of higher plants was not forthcoming until the pioneering investigation of Tanko (3), James (4, 5, 6) and Hanes (7) strongly suggested that essentially the same mechanisms might be functioning in higher plants. Subsequent analyses of plant tissue revealed systems involved in the transformations of sugars that are strikingly like those found in animal and yeast preparations. These results lend considerable weight to the value of a comparative biochemical approach to biological problems which, in this case, reveals the fact that cells from many different sources, stripped of their divergent environments, operate along strikingly similar lines.

The scheme presented in Figure 1 outlines the present status of the glycolytic cycle in animal and yeast extracts and indicates that major portions are relatively well documented in higher plants.² However, to date, no cell-free plant preparation is known which will catalyze the complete breakdown of free monosaccharides to pyruvic acid, although preparations which will carry on phosphorylative transformation of starch to pyruvic acid have been known for some time (3 to 7). Furthermore, little effort has yet been directed to the purification and characterization of the members of the glycolytic system. Q-enzyme (8), phosphorylase (9), phosphofructokinase (10), aldolase (11), and pyruvic decarboxylase (94) are in fact the only enzymes which have been carefully studied.

In recent years there has been a tendency among investigators in plant metabolism to duplicate the experiments and interpretations of workers in the fields of animal and yeast metabolism. It must be pointed out, therefore, that the glycolytic cycle will be placed in its proper position in the over-all

¹ The survey of the literature pertaining to this review was concluded in November 1951.

² Throughout the text, bracketed references in Roman numerals pertain to the enzyme systems and reactions set forth in Figure 1.

metabolism of plants only after a thorough survey has been conducted of the distribution of the glycolytic enzymes and of their participation in various phases of the life cycle of plants. Recent evidence suggests indeed that

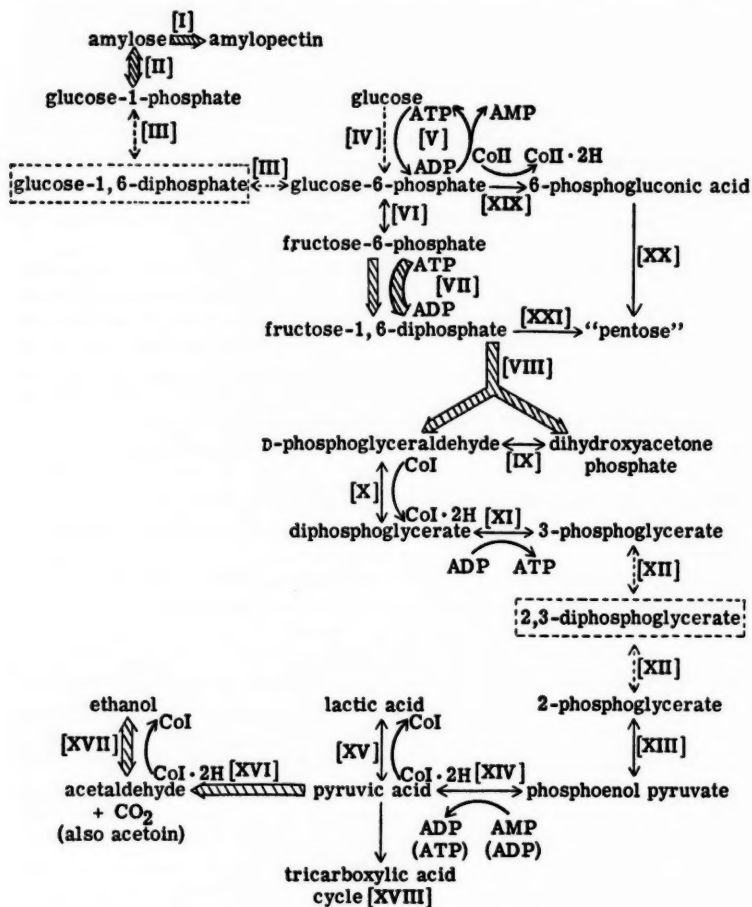


FIG. 1. Present scheme for anaerobic breakdown of carbohydrates.

hatched arrow enzyme isolated and purified from plant tissue
 solid arrow enzyme known to be present in plant tissues
 dashed arrow no evidence

(Explanation of enzymes on facing page)

whereas the glycolytic cycle is a functioning system in seeds, other systems may be responsible for carbohydrate transformations in mature leaf tissue (90, 91).

To facilitate discussion, the reaction sequences of glycolysis will arbitrarily be divided into three stages: (a) formation and phosphorolysis of starch, (b) interconversions of hexose phosphates, and (c) transformations of the C_3 sugars.

FORMATION AND PHOSPHOROLYSIS OF STARCH

Q-enzyme [1].²—After Cori *et al.* (12) had discovered phosphorylase and had demonstrated the synthesis *in vitro* of amylose, the linear α -1,4-glucosidically-linked chain, from glucose-1-phosphate, considerable effort was exerted to explain the paradox that plant as well as animal tissues synthesize the highly branched amylopectin component which in addition to the 1-4 also contains 1-6 glucosidic linkages. To explain these puzzling facts, it was assumed that another enzyme must exist which, in association with phosphorylase, forms amylopectin from glucose-1-phosphate. Therefore, when in 1943 Cori & Cori (13) presented evidence for the branching factor in liver which, in conjunction with phosphorylase, synthesized glycogen from glucose-1-phosphate, this hypothesis appeared to be essentially substantiated. At about the same time, Peat and co-workers in England independently undertook the task of elucidating this problem in plant tissues. In an elegant series of papers (14-25) they showed that in the purification of potato phosphorylase, the first ammonium sulfate fraction, which was low in phosphorylase activity and hence ordinarily discarded, contained an enzyme which had the property of a branching factor. This enzyme—called *Q-enzyme* by the English group, in distinction to phosphorylase which is referred to as *P-enzyme*—when allowed to react in the presence of phosphorylase and glucose-1-phosphate yielded a product which gave a purple-red color with iodine, was soluble in water, did not retrograde from solution, and was only partly attacked by β -amylase. End-group assay of the product by exhaustive methylation

Enzymes:

- | | |
|--|--|
| I. <i>Q-enzyme</i> | XII. phosphoglyceric mutase |
| II. phosphorylase | XIII. enolase |
| III. phosphoglucomutase | XIV. phosphoenol pyruvate transphosphorylase |
| IV. hexokinase | XV. lactic dehydrogenase |
| V. adenylkinase | XVI. carboxylase |
| VI. phosphohexoisomerase | XVII. alcohol dehydrogenase |
| VII. phosphofructokinase | XVIII. Krebs cycle enzyme system |
| VIII. aldolase | XIX. glucose-6-phosphate dehydrogenase |
| IX. triosephosphate isomerase | XX. Horecker-Cohen system |
| X. triosephosphate dehydrogenase | XXI. "aldolase" |
| XI. triosephosphate transphosphorylase | |

indicated the existence of branching chains about 20 glucose units long. It was also later shown that Q-enzyme converts linear amylose directly to amylopectin in the absence of inorganic phosphate and phosphorylase.

It was first believed by Bourne & Peat (15) that phosphorylase condensed glucose-1-phosphate to an unbranched chain which they termed pseudo-amylose, consisting of approximately twenty 1-4 linked glucose units. In the absence of Q-enzyme, pseudoamylose was simply extended to long-chain amylose molecules. In the presence of Q-enzyme, however, 1-6 linkages were established between short chains of pseudoamylose to form the branched amylopectin molecule. A second function for Q-enzyme was the scission of 1-4 linkages in long-chain amylose molecules to the shorter pseudoamylose chains, in the absence of inorganic phosphate and phosphorylase.

Recent evidence by Hobson, Whelan & Peat (25) indicates, however, that this original hypothesis has had to be revised. They prepared a series of short-chain dextrans by the controlled action of salivary amylase on amylose. To these dextrans was added Q-enzyme, and the reaction mixture was then analyzed for conversion to branched polymers. It was found that as shorter-chain dextrans were substituted for amylose in the Q-enzyme system, synthesis of the branched polysaccharide diminished sharply. It was therefore concluded that Q-enzyme was not able to join, through 1-6 linkages, dextrans containing glucose residues of less than 25 units. Rather, Q-enzyme ruptures long amylose chains at appropriate 1-4 links and forms an enzyme-substrate complex, thereby conserving the energy of the severed 1-4 glycosidic bond; it then transfers and condenses the previously split chain through 1-6 glycosidic links to random points along the long amylose chain. It appears therefore, that the scission of 1-4 links and the synthesis of 1-6 links are interdependent and occur simultaneously through a transglycosidic reaction. Supporting this conclusion is the observation of Nussenbaum & Hassid (26) who have recently prepared a series of amylo-dextrans of known chain length and have demonstrated that the minimum chain length of the dextrin serving as a substrate for Q-enzyme is between 42 and 116 glucose units, the average value being probably between 75 and 85 units. Linear dextrans below 42 glucose units in length are inert in the Q-enzyme system.

In studying the *in vitro* synthesis of amylopectin from glucose-1-phosphate, Barker *et al.* (22) have examined the relation between the product and the ratio P/Q of phosphorylase/Q-enzyme in enzyme digests. It was found that with a high P/Q ratio, the properties of the synthesized polysaccharide were those of an amylose, whereas with low P/Q ratios amylopectin synthesis predominated. With ratios between these limits, the polysaccharide formed appeared to have a molecular structure intermediate between those of amylose and amylopectin. This study is of interest in that it suggests the possible permutations and combinations of different ratios of these two enzymes which are available in the plant cell for starch synthesis.

Nussenbaum & Hassid (27) have completely confirmed the results of the Peat group and have answered some criticisms leveled at the British workers

by Beckman & Rogers (28). They have prepared amylopectin by the reaction of Q-enzyme on corn amylose and carefully studied its properties. This polysaccharide was hydrolyzed 51 per cent by crystalline β -amylase, whereas the starting substrate, corn amylose, was degraded under the same conditions to 90 per cent. Since no fatty acids were found in the purified amylopectin, Beckman & Rogers' original criticism (28) that fatty acid-amylose complexes in the enzyme digest gave a red color to iodine, thereby simulating amylopectin synthesis, is invalidated. End-group analysis by the periodate method demonstrated that the average number of glucose residues per end group of the product was 21, whereas the chain length of the parent corn amylose was 490 glucose residues per end group. Reducing value measurements proved that the product could not consist of short-chain degraded amylose molecules. Finally, Cori & Illingworth (29) analyzed the synthetic product of Nussenbaum & Hassid by their elegant amylo-1,6-glucosidase end-group method and obtained a value of 20 glucose units per chain length, which is in excellent agreement with chemical findings of Nussenbaum & Hassid. Further, the synthetic amylopectin was degraded 34 per cent to glucose-1-phosphate by phosphorylase in agreement again with the idea that the product has 1-6 links. Successive degradation by both amylo-1,6-glucosidase and phosphorylase clearly demonstrated that the polysaccharide is tree-like or multibranched in structure and not singly branched or laminated as formulated by Peat and his co-workers. This mass of evidence overwhelmingly confirms the results of this group and firmly establishes Q-enzyme as an essential factor in the biosynthesis of 1-6 links in polysaccharides.

It has been recently reported that by a combination of alcohol and ammonium sulfate fractionations, the crystalline form of Q-enzyme was obtained (30). Free of phosphorylase, maltase, phosphatases, and β -amylase, it contained a small constant amount of α -amylase activity. The elucidation of the role of this residual activity in the crystalline form of Q-enzyme (assuming, of course, a one-component protein system) will be of considerable interest.

While the British workers were developing the concept of Q-enzyme action, Bernfeld & Meutémédian (31a) in Switzerland announced the discovery of potato "isophosphorylase." Briefly, they claimed that glucose-1-phosphate formed by conversion of amylose in the presence of $\text{PO}_4^{=}$ and phosphorylase was utilized by "isophosphorylase" to form 1-6 linked branches phosphorylatically. In addition, glucose-1-phosphate was further condensed by phosphorylase to the newly synthesized 1-6 stubs to form the extended branches of native amylopectin. It should be noted that these authors actually never isolated a compound that could be studied by standard chemical procedures. They dealt with a crude digest which was characterized only by its color reaction with iodine and by the hydrolysis limit with β -amylase. Attempts to repeat this experiment by three separate groups of workers, namely Bailey & Whelan (24), Nussenbaum & Hassid (27), and K. H. Meyer & co-workers (31b), yielded negative results. No phosphorolytic enzyme having the prop-

erties ascribed to "isophosphorylase" could be detected. It therefore appears that no such enzyme exists; actually it probably is a mixture of phosphorylase and amylases. It is very unfortunate that Bernfeld, aware of this situation, should have included in his recent review (31c) the highly questionable "isophosphorylase" with the well-known phosphorylases and transglycosidases, whose roles have been well established in the synthesis of starch-like polysaccharides.

Phosphorylase [II].—The discovery by Hanes (32) of phosphorylase in plant tissues initiated a large number of intensive investigations on the nature and role of this enzyme in plant tissue. As yet no other enzyme in the glycolytic cycle of plants has enjoyed such attention. Since recent reviews (33, 34) have carefully described the kinetics, properties, and preparation of this enzyme, only the latest advances will be discussed here.

Phosphorylase has now been found in pea (7), waxy maize (35), sweet potato (36), potato (32, 37), pumpkin (38), sugar beet (39), lima bean (37), barley (40), jackbean (41), and broad bean (23). However, Porter (42) has recently pointed out some difficulties in analyzing for phosphorylase activity in crude extracts of grain. It was found that phosphorylase activity in crude aqueous extracts of wheat, oats, barley, and rye was masked to a considerable extent. When these extracts were added to purified potato phosphorylase, considerable inhibition was observed which did not occur when boiled extracts were added. According to Porter, at least three factors are involved: (a) the rather low pH of the initial extract which leads to some instability of the enzyme, (b) the large amounts of β -amylase present in the extracts (β -amylase would not only degrade the starch synthesized from glucose-1-phosphate but would also probably destroy the starch primer essential for the initiation of the synthesis), and (c) the crude extracts possess an unknown inhibitory factor which cannot be explained on the basis of the above two effects.

Yin & Sun (43) have continued their histochemical analysis of plant tissues and have found phosphorylase activity most intense in the rootcap of soybeans, less in the root tip and lateral buds, still less in the hypocotyl and stem tip, and very little in cotyledons. In leaves the enzyme was localized in chloroplasts of guard cells and of mesophyll cells. Dyar (44), employing similar techniques with pea root tips, found that starch synthesis was most active in the root cap cells, was also abundant in meristematic cells and procambium, and less so in the cortex. Stocking (45), again using histochemical tests for the intracellular localization of phosphorylase in sections of destarched leaves of potato, chard, and sunflower, could not demonstrate that the enzyme is located within chloroplasts. Cells in thin sections or cells near cut surfaces of leaf segments impregnated with glucose-1-phosphate showed the development of iodine-staining polysaccharide granules in the nonplastid cytoplasm. Gentle homogenization of leaves in sucrose followed by centrifugation to remove plastids and plastid fragments yielded a supernatant liquid relatively free of chlorophyll but containing the largest amount of enzyme activity. It was concluded by Stocking that if leaf phosphorylase is

localized within plastids in normal leaves this association is extremely labile.

Although histochemical techniques are of considerable importance in evaluating the distribution of a given enzyme in the cell, employment of these techniques in a study of the effects of various reagents and inhibitors on the *in vivo* activity of the enzyme must be approached with considerable care so far as the interpretation of the data is concerned. The work of Stocking clearly points out the difficulties involved in this type of approach.

In investigating the role of carbohydrate primers of potato phosphorylase, Bourne *et al.* (46) have confirmed the earlier findings of Cori *et al.* (47) and others (48) that the enzyme functions only by the addition of glucose units derived from glucose-1-phosphate to a pre-existing chain of 1-4 linked glucose units. The "template" chain may be a straight chain like amylose which has low primer activity or a highly branched system like amylopectin with high activating effects. Weibull & Tiselius (49) had earlier separated dextrans by adsorptive analysis into fractions corresponding to tri-, tetra-, penta-, and hexasaccharides and found that each of these fractions had priming activity, indicating that the minimum length for a priming chain is three glucose units. Bailey *et al.* (50) have recently applied the method of Whistler & Burso (51) to a mixture of dextrans derived from amylose by acid hydrolysis and obtained pure specimens of glucose, maltose, maltotriose, maltotetraose, maltopentaose, and maltohexaose. It was found that maltotriose was the first member of the series which was active, although it was only weakly so. Since the authors eliminated the possibility that the more active higher members of the series were present in trace amounts in the maltotriose sample, it appears that the minimum length for a primer molecule must be that of three glucose units, linked together by 1-4 glucosidic bonds.

Sumner *et al.* (41) have purified jackbean phosphorylase approximately one thousandfold. As with other plant phosphorylases, cysteine and adenylic acid are not activators for the enzyme. The authors state that amylose was found to be an efficient activator whereas amylopectin was not. Unfortunately no evidence was cited to support this interesting observation which would suggest a sharp difference in primer requirement between jackbean phosphorylase and all other plant phosphorylases.

Katz & Hassid (52) have made the important observation that when phosphate is replaced by arsenate in the scission of starch by potato phosphorylase, glucose rather than glucose-1-arsenate accumulates; arsenate remains in the inorganic form. The authors conclude that a very labile glucose-1-arsenate may be formed in an "arsenolytic" breakdown of starch, and that it immediately hydrolyzes spontaneously to yield inorganic arsenate and free glucose. Attempts to isolate this postulated intermediate were unsuccessful. The kinetics of the arsenolytic breakdown process of amylose and amylopectin were found to be similar to that of β -amylase hydrolysis. This arsenolytic reaction can now be added to the growing list of reactions which are radically modified by replacement of phosphate with arsenate in an enzyme system. This list includes the oxidation of phosphoglyceraldehyde by triose

phosphate dehydrogenase (53), the enzymic decomposition of acyl phosphates (54), the oxidation of butyric acid by *Clostridium kluyveri* extracts (55), and glutamine transamidation reaction of glutamyltransphorase (56).

It was demonstrated (57) that the sucrose phosphorylase of *Pseudomonas saccharophila* acts as a transglucosidase by transferring glucose units from one disaccharide to another. A sensitive test for such transferring activity was suggested (57) when it was observed that a rapid interchange of phosphate occurred between the organic and inorganic fractions without liberation of any glucose when glucose-1-phosphate and radioactive inorganic phosphate (P^{32}) were added in the absence of any glucose acceptor. Cohn & Cori (58) examined potato phosphorylase for similar transglycosidase activity by incubating radioactive inorganic phosphate (P^{32}) and glucose-1-phosphate with the enzyme in the absence of a polysaccharide as acceptor. Since there was no incorporation of radioactivity in the organic phosphate fraction, it was concluded that phosphate transfer by potato phosphorylase differed radically from that of sucrose phosphorylase.

INTERCONVERSION OF HEXOSE PHOSPHATES

Little direct evidence is at present available concerning the enzymes involved in the conversion of glucose-1-phosphate to fructose diphosphate. Tanko in 1936 (3) demonstrated the transformation of starch by pea meal extracts to small amounts of glucose-6-phosphate, fructose-6-phosphate, and rather large amounts of fructose diphosphate. Hanes (7) in 1940 confirmed the results of Tanko and isolated small amounts of glucose-1-phosphate, fructose-6-phosphate, and again fairly large amounts of fructose diphosphate from pea meal extracts containing starch as the initial substrate. Axelrod *et al.* (59) have recently repeated these experiments employing radioactive inorganic phosphate (P^{32}) and paper chromatographic techniques as refinements and have observed substantially the same products of breakdown. The conventional conversion enzymes which catalyze the formation of phosphorylated hexoses from starch therefore appear to be present in plant extracts.

It is unfortunate, though, that no concerted effort has been made to isolate the component enzymes. For example, phosphoglucomutase [III] has as yet not been isolated and characterized. Moreover, although Leloir and his co-workers (104) have demonstrated the function of glucose-1,6-diphosphate in the phosphoglucomutase reaction in yeast and animal extracts, there is no evidence as yet to justify the extension of their conclusions to plant phosphoglucomutase. As a result, nothing can be said concerning the role of glucose diphosphate in the reaction glucose-1-phosphate \rightarrow glucose-6-phosphate.³ However, Sisakyan and Kobayakova (6) have demonstrated

³ Very recently C. C. Cardini (105) has extracted phosphoglucomutase from jack-bean seeds. The enzyme requires glucose-1,6-diphosphate as well as Mg^{++} and cysteine for full activity. Addition of ATP to glucose-1-phosphate and seed extract results in synthesis of glucose-1,6-diphosphate. Thus, the last remaining missing part of the complete glycolytic cycle in plants has been found.

phosphoglucumutase activity in tomato leaf plastids. When glucose-1-phosphate was incubated with the plastid preparation in cysteine buffer, an equilibrium mixture of glucose-1-phosphate and another phosphorylated hexose was obtained. This phosphate derivative, when isolated as the lead salt had properties identical to those of a known sample of glucose-6-phosphate.

Even less is known about hexokinase [IV] in plants. Indirect evidence has been presented by Barrón *et al.* (61) who observed that glucose utilization in washed potato slices was slightly inhibited by 10^{-2} *M* DL-glyceraldehyde. Since Rudney (62) had earlier demonstrated an inhibitory effect on animal and yeast hexokinase by this compound, it was concluded that the observed inhibition in potato slices involved hexokinase inactivation. Rudney found that muscle hexokinase was inhibited 100 per cent by 2×10^{-3} *M* DL-glyceraldehyde, although with yeast hexokinase only partial inhibition was observed at 10^{-2} *M*. Because of the considerable variation in the degree of inhibition by DL-glyceraldehyde, depending on the source of hexokinase, little if any conclusion can be drawn concerning its effects on plant hexokinase. Evidence indicating the presence of a hexokinase in spinach leaf brei (63) and in *Avena* coleoptile (64) is also of a very indirect nature. After glucose and ATP were incubated with a spinach leaf brei preparation at 30° for 24 hr., a compound having the properties of fructose diphosphate was isolated by barium fractionation of the reaction mixture. However, in 1947, Wildman & Bonner (65) demonstrated a very active adenosine triphosphatase and fructose diphosphatase in similar spinach bries. It is apparent, therefore, that after brief incubation, little if any added ATP was available for the phosphorylation of glucose in such extracts. Consequently, the results with spinach leaf extracts (63) and probably with *Avena* coleoptile extracts (64) cannot be interpreted as demonstrating hexokinase activity. However, strong evidence for hexokinase activity in cell-free plant extracts has been observed recently by Millerd *et al.* (66). They have made the important discovery that when plant mitochondria from etiolated mung bean seedlings are incubated with sucrose (as a source of glucose and fructose), radioactive inorganic phosphate (P^{32}), and a Krebs cycle intermediate, radioactive hexose phosphate can be isolated. This would imply that, in plants, hexokinase activity is not associated with soluble protein systems of the cytoplasm but with particulate matter, presumably mitochondria. Future investigations should elucidate the precise nature of the distribution of hexokinase activity in the plant. If such activity is found to be associated only with mitochondrial particles, experiments must be designed to relate such mitochondrial activity with the activities of those glycolytic enzymes known to be in solution in the cytoplasm.

In animal tissues it was shown sometime ago that ADP (adenosine diphosphate) can dismute to form AMP (adenylic acid) and ATP (adenosine triphosphate). The enzyme responsible for this catalysis has been called myokinase and has lately been found in kidney and liver (67), as well as in muscle (68), in animal mitochondria (69) and in yeast extracts (70). In the

reviewer's laboratory (71) preliminary results strongly indicate that a similar type of dismutation takes place in plant material. Employing paper chromatographic analysis, it has been found that ATP and AMP are formed from ADP in extracts of acetone powders of pumpkin and manroot. Because of the presence of an active adenylic acid-5-phosphatase, AMP was rapidly dephosphorylated to adenosine in pumpkin leaf extracts but not manroot extracts. Using manometric technique, it was also shown that in a yeast hexokinase-ADP-glucose test system, plant extracts could substitute for rabbit muscle myokinase by converting ADP to ATP. These results may explain the dual function of ADP and ATP in activating purified pumpkin glutamyltransphorase, the enzyme which catalyzes the exchange reaction between $N^{15}H_3$ and amide NH_3 in glutamine (56). It would appear as though plant myokinase (a more appropriate term would be adenykinase [V]) is present in preparations of glutamyltransphorase and is catalyzing the transformation of ATP to ADP which may be the active cofactor. Independent kinetic studies indicate this reasoning to be correct (71).

Although phosphohexoisomerase [VI] activity has been observed by Tanko (3) and by Hanes (7) in pea meal extracts, no attempt has yet been made to isolate and characterize the enzyme. Nevertheless, Somers & Cosby (72) employing dialyzed water extracts of pea seeds found that 67 per cent of fructose-6-phosphate disappeared in 80 min. while an equivalent amount of glucose-6-phosphate was formed.

Much more evidence is available for the occurrence of phosphofructokinase [VII]. The early work of Tanko and Hanes strongly suggested the presence of this enzyme in pea meal extracts. Recent investigations by Axelrod *et al.* (10) have fully documented this enzyme system. The system catalyzes the formation of fructose diphosphate from fructose-6-phosphate and ATP. It has been concentrated and purified by fractional precipitation with ammonium sulfate. The Michaelis constants with respect to fructose-6-phosphate and ATP respectively are approximately $7 \times 10^{-3} M$ and $2 \times 10^{-3} M$. Magnesium or manganese ions are required for full activity; cupric and zinc ions are inhibitory. Fluoride ion at 0.01 M concentration is without effect.

TRANSFORMATIONS OF C_3 SUGARS

Of the many enzymes participating in the glycolytic cycle, those more thoroughly studied are involved in the breakdown of fructose diphosphate (FDP) to pyruvic acid, or acetaldehyde and CO_2 . In these processes, James (4) was one of the first investigators to elucidate the fermentative breakdown of FDP in plants. Working with preparations of barley seedling extracts, he was able to show that addition of 0.025 M fluoride caused the accumulation of an ester from the breakdown of FDP which by its hydrolysis properties was suspected of being phosphoglyceric acid (PGA). In the absence of fluoride both FDP and PGA were converted to pyruvate, which accumulated if 1-naphthol-2-sulfonic acid was added to reduce carboxylase activity.

Pyruvate was isolated as the 2,4-dinitrophenylhydrazone. An analogous system catalyzing the breakdown of FDP to the triose phosphate stage has been found in pollen extracts (73).

Evidently, then, all the enzymes that participate in the $C_6 \rightarrow C_3 \rightarrow C_2$ conversion reactions appear to be present to some degree in some plant tissues. Isolation and characterization of these enzymes have unfortunately lagged considerably. Recent work has, however, made it possible to define some of these enzymes more thoroughly.

Aldolase [VIII]—While this enzyme has been identified in plant tissues since 1934 (74), no precise knowledge of its kinetics or properties was available until 1948. Stumpf (75) isolated and studied the properties of pea aldolase prepared by a series of ammonium sulfate, isoelectric, and acetone fractionation procedures which yielded a water-clear solution purified some 90-fold and stable for a considerable period of time if kept frozen. The Michaelis constant is approximately 8×10^{-4} M at pH 8.5 and at a temperature of 31° which suggests that its affinity for the substrate, FDP, is some 10-fold higher than that of animal aldolase which has a Michaelis constant of 9×10^{-3} M. The equilibrium constant K was found to be 1.15×10^{-4} at 31° in a borate buffer at pH 8.5 which agrees quite well with the K value of 0.9×10^{-4} M at 31° obtained for muscle aldolase by Meyerhof (76). In contrast to the findings of Warburg with yeast aldolase (77), and Knox (78) and Gunsalus (79) with bacterial aldolase, but in agreement with the results of Taylor *et al.* with crystalline muscle aldolase (80), no divalent cation was found as an essential cofactor. However, in recent work (81) with plants grown in zinc-deficient nutrient solution, aldolase activity was found to fall sharply. Addition of zinc to the medium caused the reappearance of the enzymatic activity. Whether this effect is of a direct or a more indirect nature requires further investigation. In employing tissues obtained from plants grown in metal-deficient media, it is always difficult to relate changes in enzymatic activity to requirements of metals as cofactors because of gross complications which affect the many enzyme systems of the deficient plant.

The distribution of aldolase in higher plants was studied by Tewfik & Stumpf (82) and was found to be ubiquitous. Of 29 different species studied, ranging through fungi, ferns, conifers, monocotyledons, and dicotyledons, all contained measurable amounts of activity. The presence of the enzyme in the pea plant was studied with respect to the life cycle of the plant. It was detected in measurable amounts in every gross part of the plant from germination to the final formation of new seeds in the green pod. In the roots of seedlings the highest activity was found in the meristematic region. High activity was found consistently in leaf tissue. Finally, after separation of tomato leaf and sugar beet leaf tissue into the cytoplasmic and particulate fractions, the latter containing broken chloroplasts, grana, and mitochondrial particles, it was found that no activity was associated with the particulate fraction, whereas considerable activity was localized in the nonparticulate cytoplasmic fraction. The presence of aldolase in the cytoplasmic fraction in

leaf tissue implies that in the leaf, sugar synthesis via photosynthesis is not an exclusive property of the chloroplast (assuming that hexoses are synthesized in part by the glycolytic system); actually a division of labor occurs by which part of the total synthesis takes place at the site of chlorophyll-catalyzed reactions, while part occurs in the cytoplasmic layer of plant cells.

Because of the reversible nature of the reaction catalyzed by aldolase, Meyerhof *et al.* (83) were able to show that the addition of any aldehyde to aldolase and dihydroxyacetone phosphate would result in the formation of new sugars. Thus the addition of a C_2 aldehyde to the C_3 triose phosphate would form a C_5 sugar. It was therefore suggested by Tewfik & Stumpf (82) that because of the widespread distribution of the enzyme in plants and because barley, oats, and corn, cereal plants known for high pentosan contents, had the highest activity of the 29 species tested, aldolase may conceivably be associated with pentose synthesis in plants. Recently Forrest, Hough & Jones (84) have reported that pea aldolase, when added to fructose diphosphate and glycollic-aldehyde, catalyzes the formation of an optically active ketopentose containing largely D-xyloketose. Further investigation will reveal whether there is a pentoisomerase in plant tissues which can catalyze the conversion of the ketopentoses formed in this type of reaction to the normally occurring aldopentoses. Hassid and co-workers (85) have independently observed results similar to those of Forrest *et al.* These independent findings tend to support the earlier prediction and place aldolase or an enzyme of similar properties in a key position in sugar conversion in higher plants. It remains to be shown, however, whether highly purified aldolase can form the pentoses mentioned above or whether the complete system actually involves a group of similar "aldolase-like" enzymes.

Mention should here be made of the important demonstration by Conn & Vennesland (86) of glucose-6-phosphate dehydrogenase activity in a variety of plants [XIX]. They also present evidence which suggests a system similar to that in yeast or bacterial cells whereby D-glucose-6-phosphate is oxidatively decarboxylated to D-ribose (87, 88). Therefore, pentose synthesis in plants may proceed from two directions, namely from glucose phosphate and from triose phosphate plus glycollic aldehyde.

Isomerase [IX].—Although this enzyme has not been purified from plant sources, its presence is clearly indicated by balance studies carried out in investigating the breakdown of FDP to PGA in pea seed extracts (89).

Triose phosphate dehydrogenase system [X].—Using pea acetone powder extracts, Stumpf (89) was able to prove conclusively that this dehydrogenase is an active component in the breakdown of FDP by pea extracts. Acceleration of acid formation by arsenate, reduction of Co I in the presence of either phosphate or arsenate but absence of reduction when these anions were not added, iodoacetamide and copper inhibition, and the formation of PGA were the observations which indicated the participation of triose phosphate dehydrogenase in these preparations.

However, recent work has thrown some doubt on the conclusions con-

cerning the role of triose phosphate dehydrogenase. Tewfik & Stumpf (90) were not able to demonstrate triose phosphate dehydrogenase activity in leaf tissues. A search was then made to determine which enzymes of the glycolytic cycle were missing in leaf extracts. It was shown that aldolase and triose phosphate isomerase were present throughout the life cycle of the plant tissue, but it was also found that while pea seed extracts could readily reduce Co I on the addition of FDP (aldolase in the preparation cleaving FDP to triose phosphate), leaf preparations were inactive. Studies on the pea plant furthermore indicated that there is a gradual decrease in glycolytic activity upon increase in age of the plant from the first to the eighth day. On the sixth day no activity could be detected in the seedling although activity was still found in the attached cotyledons. Increase in oxygen uptake by leaf extracts in the presence of FDP was not affected by additions of DPN, arsenate, or iodoacetic acid.

It was therefore concluded that in leaf tissues the conventional triose phosphate dehydrogenase system is missing. In this connection, mention should be made of the interesting observation of Goddard & Meeuse (2) that in the very young pea seedlings the fermentation to respiration ratio (F:R) was high, whereas in older seedlings the ratio fell considerably. It would be of some interest to design a series of experiments interrelating the results of Tewfik & Stumpf with those of Goddard & Meeuse.

In explaining the utilization of FDP by leaf tissue, Tewfik & Stumpf (91) have demonstrated the existence of a system in pea leaf lyophilizates which oxidizes FDP through a series of as yet undefined reactions rather than through the conventional glycolytic pathway. These oxidative reactions require flavinadeninedinucleotide, ATP, and presumably ascorbic acid, as cofactors. PGA is not a product of the oxidation. For each mole of FDP consumed, one mole of oxygen is taken up. The FDP system is not inhibited by fluoride, cyanide, or iodoacetamide, indicating that enolase, cytochrome oxidase, ascorbic acid oxidase, and triose phosphate dehydrogenase are not involved.

At present little can be said concerning the preferential mode of breakdown of FDP in mature leaf tissue. Since available evidence indicates the disappearance of triose phosphate dehydrogenase in mature tissue, another system must assume the role of the oxidation of triose phosphate. FDP may be degraded to the triose phosphate stage by aldolase at which point a dismutation could take place to yield indirectly PGA which is then degraded via the conventional $\text{PGA} \rightarrow \text{pyruvic acid}$ enzyme systems. In contrast, FDP may be oxidized via a system independent of triose phosphate and the $\text{PGA} \rightarrow \text{pyruvate}$ enzyme systems to C_3 or C_2 fragments. The elucidation of these problems will depend in part on the characterization of the oxidation product of Tewfik's system.

A novel type of respiration system was recently studied by James & Beevers (92). They were able to prepare water extracts of *Aroid* spadices of unusual high respiratory activity. FDP considerably stimulated oxygen up-

take which was inhibited by iodoacetamide. Neither cyanide nor carbon monoxide inhibited oxygen uptake, suggesting that metalloenzymes such as the polyphenol oxidase and cytochrome oxidase were not serving as terminal oxidases. Since riboflavin stimulated oxygen uptake, it was suggested that the spadix system probably involved starch breakdown via FDP, the oxidation stage depending on a flavoprotein acting as the coupling system with atmospheric oxygen.

PGA → Pyruvic acid system.—Evidence for the participation of the conventional glycolytic enzymes in the breakdown of PGA to pyruvic acid has been presented by James (46) and recently by Stumpf (89), and Tewfik & Stumpf for pea seeds and leaf tissues (90, 91). It was shown (89) that when PGA was added to 2-hr. dialyzed pea seed extracts, little if any CO_2 formation could be observed from the decarboxylation of pyruvic acid. However, on addition of magnesium, cocarboxylase, and adenylic acid, activity could be fully restored. The addition of fluoride virtually inhibited CO_2 formation. These data can be interpreted to mean (a) that an enolase [XIII] is present which is sensitive and inhibited by fluoride and requires magnesium, (b) that a phosphopyruvic transphosphorylase [XIV] is present and transfers energy rich phosphate bonds from phosphoenolpyruvic acid to adenylic acid as the acceptor, and (c) that a carboxylase [XVI] is activated by cocarboxylase and magnesium. This same multienzyme system is active in pea leaf preparations. Life cycle studies in peas revealed little over-all change in the pattern of its activity (90).

In these studies it was observed that the phosphoenolpyruvic transphosphorylase system (89) was rather labile since 12-hr. dialysis of pea seed extracts resulted in complete inactivation. Addition of boiled undialyzed extracts did not reactivate the enzyme. Earlier work indicated that the triosphosphate transphosphorylase [XI] was inactive in pea seeds, but recent studies by Newcomb (93) suggest that the system is present in extracts of pea seeds.

Since the keto acid decarboxylases are being reviewed by Vennesland in this volume, no discussion of these important enzymes is necessary. So far as pyruvic acid, the end product of glycolysis, is concerned, at least six reaction paths have been demonstrated for it in plant tissues. First, it can be reduced to lactic acid in potato slice by lactic dehydrogenase [XV] according to the work of Barrón *et al.* (61); second, it can be both decarboxylated to acetaldehyde and CO_2 and condensed to acetoin by wheat germ carboxylase [XVI] which was described by Singer (94); third, it can be reduced directly to ethanol by alcohol dehydrogenase [XVII] (89); fourth, it can be oxidized to carbon dioxide and water by a plant mitochondrial system [XVIII] in the presence of a Krebs Cycle intermediate (66); fifth, it can be coupled to known transaminase systems (95); and sixth, it can react with carbon dioxide in the presence of the "malic" enzyme to form malic acid (96).

The controversial subject of the relation between glycolysis and photosynthesis has been omitted in this review since the problem has been thor-

oughly discussed in a series of excellent papers in the *Annual Review of Plant Physiology*, volumes 1 to 3. However, the recent observations of the Ochoa, Gaffron, and Arnon groups (97 to 99) will undoubtedly throw much light on the problem in the near future.

It is a pleasant task to end this discussion by listing a series of extremely useful reviews on the general subject of glycolysis. Bonner's contribution in the form of his outstanding *Plant Biochemistry* focuses attention on the many problems in the new field of plant biochemistry. Hassid and co-workers (33, 34) have thoroughly reviewed the subject of phosphorylases; Hehre (100) and Peat (101) have discussed with authority the many aspects of polysaccharide transformations in plants and bacteria; and Hough & Jones (102) have written, from the organic chemist's point of view, a provocative paper concerning pentose and hexose biosynthesis. Two important books have been published, the first being an encyclopedic work, entitled *The Enzymes* and edited by Sumner & Myrbäck (103), in which have been collected excellent chapters on a large number of enzymes including the glycolytic enzymes, and the second entitled *Phosphorus Metabolism* (104), in which have been gathered outstanding papers on the many facets of phosphate metabolism by leaders in the field.

LITERATURE CITED

1. Hassid, W. Z., and Putman, E. W., *Ann. Rev. Plant Physiol.*, **1**, 109 (1950)
2. Goddard, David R., and Meeuse, B. J. D., *Ann. Rev. Plant Physiol.*, **1**, 207 (1950)
3. Tanko, B., *Biochem. J.*, **30**, 692 (1936)
4. James, W. O., James, G. M., and Bunting, A. H., *Biochem. J.*, **35**, 588 (1941)
5. James, W. O., Heard, C. R. C., and James, G. M., *New Phytologist*, **43**, 62 (1944)
6. James, G. W., and James, W. O., *New Phytologist*, **39**, 266 (1940)
7. Hanes, C. S., *Proc. Roy. Soc. (London)*, [B]**128**, 421 (1940)
8. Haworth, W. N., Peat, S., and Bourne, E. J., *Nature*, **154**, 236 (1944)
9. Hanes, C. S., *Proc. Roy. Soc. (London)*, [B]**129**, 174 (1940)
10. Axelrod, B., Saltman, P., Bandurski, R. S., and Baker, R. S., *Proc. Am. Soc. Plant Physiol., Western Sect.*, **1** (June, 1951) (Abstracts)
11. Stumpf, P. K., *J. Biol. Chem.*, **176**, 233 (1948)
12. Cori, C. F., Schmidt, G., and Cori, G. T., *Science*, **89**, 464 (1939)
13. Cori, G. T., and Cori, C. F., *J. Biol. Chem.*, **151**, 57 (1943)
14. Haworth, W. N., Peat, S., and Bourne, E. J., *Nature*, **154**, 236 (1944)
15. Bourne, E. J., and Peat, S., *J. Chem. Soc.*, 877 (1945)
16. Bourne, E. J., Macey, A., and Peat, S., *J. Chem. Soc.*, 882 (1945)
17. Peat, S., Bourne, E. J., and Barker, S. A., *Nature*, **161**, 127 (1948)
18. Barker, S. A., Bourne, E. J., and Peat, S., *J. Chem. Soc.*, 1705 (1949)
19. Barker, S. A., Bourne, E. J., and Peat, S., *J. Chem. Soc.*, 1712 (1949)
20. Barker, S. A., Bourne, E. J., Wilkinson, I. A., and Peat, S., *J. Chem. Soc.*, 84 (1950)
21. Barker, S. A., Bourne, E. J., Wilkinson, I. A., and Peat, S., *J. Chem. Soc.*, 93 (1950)
22. Barker, S. A., Bourne, E. J., Peat, S., and Wilkinson, I. A., *J. Chem. Soc.*, 3022 (1950)
23. Hobson, P. N., Whelan, W. J., and Peat, S., *J. Chem. Soc.*, 3566 (1950)
24. Bailey, J. M., and Whelan, W. J., *J. Chem. Soc.*, 3573 (1950)
25. Hobson, P. N., Whelan, W. J., and Peat, S., *J. Chem. Soc.*, 596 (1951)
26. Nussenbaum, S., and Hassid, W. Z. (Unpublished data)
27. Nussenbaum, S., and Hassid, W. Z., *J. Biol. Chem.*, **190**, 673 (1951)
28. Beckmann, C. O., and Roger, M., *J. Biol. Chem.*, **190**, 467 (1951)
29. Cori, G. T., and Illingworth, B., *J. Biol. Chem.*, **190**, 679 (1951)
30. Gilbert, G. A., and Patrick, A. D., *Nature*, **165**, 573 (1950)
31. (a) Bernfeld, P., and Meutemedian, A., *Helv. Chim. Acta*, **31**, 1724, 1735 (1948);
(b) Meyer, K. H. (Private communication; also see ref. 31c, p. 419); (c) Bernfeld, P., *Advances in Enzymol.*, **12**, 379 (1951)
32. Hanes, C. S., *Proc. Roy. Soc. (London)*, [B]**129**, 174 (1940)
33. Hassid, W. Z., Doudoroff, M., and Barker, H. A., *The Enzymes*, (2)I, 1014 (Sumner, J. B., and Myrback, K., Eds., Academic Press, New York, N. Y., 1951)
34. Hassid, W. Z., and Doudoroff, M., *Advances in Enzymol.*, **10**, 123 (1950)
35. Bliss, L., and Naylor, N. M., *Cereal Chem.*, **23**, 177 (1946)
36. Inoue, Y., and Onodera, K., *Repts. Inst. Chem. Researches, Kyoto Univ.*, **15**, 70 (1946)
37. Green, D. E., and Stumpf, P. K., *J. Biol. Chem.*, **142**, 355 (1942)
38. Inoue, Y., and Onodera, K., *Repts. Inst. Chem. Researches, Kyoto Univ.*, **16**, 29 (1947)

39. Kursanov, A. L., and Pavlinova, O., *Biokhimiya*, **13**, 378 (1948)
40. Porter, H. K., *Biochem. J.*, **45**, xxxvii (1949)
41. Sumner, J. B., Chou, T. C., and Bever, A. T., *Arch. Biochem.*, **26**, 1 (1950)
42. Porter, H. K., *Biochem. J.*, **47**, 476 (1950)
43. Yin, H. A., and Sun, C. N., *Plant Physiol.*, **24**, 103 (1949)
44. Dyar, M. T., *Am. J. Botany*, **37**, 786 (1950)
45. Stocking, R., *Proc. Am. Soc. Plant Physiol., Western Sect.*, **2** (June, 1951) (Abstracts)
46. Bourne, E. J., Sitch, D. A., and Peat, S., *J. Chem. Soc.*, 1448 (1949)
47. Cori, C. F., Cori, G. T., and Green, A. A., *J. Biol. Chem.*, **151**, 39 (1943)
48. Swanson, M. A., and Cori, C. F., *J. Biol. Chem.*, **172**, 815 (1948)
49. Weibull, C., and Tiselius, A., *Arkiv Kemi, Mineral. Geol.*, [A]19, No. 19 (1945)
50. Bailey, J. M., Whelan, W. J., and Peat, S., *J. Chem. Soc.*, 3692 (1950)
51. Whistler, R. L., and Durso, D. F., *J. Am. Chem. Soc.*, **72**, 677 (1950)
52. Katz, J., and Hassid, W. Z., *Arch. Biochem.*, **30**, 272 (1951)
53. Warburg, O., and Christian, W., *Biochem. Z.*, **303**, 40 (1939)
54. Stadtman, E. R., and Barker, H. A., *J. Biol. Chem.*, **184**, 769 (1950)
55. Kennedy, E. P., and Barker, H. A., *J. Biol. Chem.*, **191**, 419 (1951)
56. Stumpf, P. K., Loomis, W. D., and Michelson, C., *Arch. Biochem.*, **30**, 126 (1951)
57. Doudoroff, M., Barker, H. A., and Hassid, W. Z., *J. Biol. Chem.*, **160**, 725 (1947)
58. Cohn, M., and Cori, G. T., *J. Biol. Chem.*, **175**, 89 (1948)
59. Axelrod, B., Bandurski, R. S., and Saltman, P., *Federation Proc.*, **10**, 158 (1951)
60. Sisakyan, N. M., and Kobyakova, A. M., *Doklady Akad. Nauk S.S.S.R.*, **67**, 703 (1949)
61. Barrón, E. S. G., Link, G. K. K., Klein, R. M., Michel, B. E., *Arch. Biochem.*, **28**, 377 (1950)
62. Rudney, H., *Arch. Biochem.*, **23**, 67 (1949)
63. Bonner, J., and Wildman, S. G., *Arch. Biochem.*, **10**, 497 (1946)
64. Bonner, James, *Arch. Biochem.*, **17**, 311 (1948)
65. Wildman, S. G., and Bonner, J., *Arch. Biochem.*, **14**, 381 (1947)
66. Millerd, A., Bonner, J., Axelrod, B., and Bandurski, R., *Proc. Natl. Acad. Sci.*, **37**, 855 (1951)
67. Barkalis, S. S., and Lehninger, A. L., *J. Biol. Chem.*, **190**, 339 (1951)
68. Colowick, S. P., and Kalckar, H. M., *J. Biol. Chem.*, **148**, 117 (1943)
69. Kielley, W. W., and Kielley, R. K., *J. Biol. Chem.*, **191**, 485 (1951)
70. Kornberg, A. (Unpublished data)
71. Loomis, W. D., and Stumpf, P. K. (Unpublished data)
72. Somers, G. F., and Cosby, E. L., *Arch. Biochem.*, **6**, 295 (1945)
73. Okunuki, K., *Acta Phytchim. (Japan)*, **11**, 27, 65, 249 (1939)
74. Baba, T., *Biochem. Z.*, **275**, 248 (1934)
75. Stumpf, P. K., *J. Biol. Chem.*, **176**, 233 (1948)
76. Meyerhof, O., and Junowicz-Kocholaty, R., *J. Biol. Chem.*, **149**, 71 (1943)
77. Warburg, O., and Christian, W., *Biochem. Z.*, **314**, 149 (1943)
78. Knox, W. E., Stumpf, P. K., Green, D. E., and Auerbach, V. H., *J. Bact.*, **55**, 451 (1948)
79. Bard, R. C., and Gunsalus, I. C., *J. Bact.*, **59**, 387 (1950)
80. Taylor, J. F., Green, H. A., and Green, G. T., *J. Biol. Chem.*, **173**, 591 (1948)
81. Quinlan-Watson, T. A. F., *Nature*, **167**, 1033 (1951)
82. Tewfik, S., and Stumpf, P. K., *Am. J. Botany*, **36**, 567 (1949)

83. Meyerhof, O., Lohmann, K., and Schuster, P., *Biochem. Z.*, **286**, 301 (1936)
84. Forrest, R. S., Hough, L., and Jones, J. K. N., *J. Am. Chem. Soc.* (In press)
85. Hassid, W. Z., Borg, R. J., and Stumpf, P. K. (Unpublished data)
86. Conn, E. E., and Vennesland, B., *J. Biol. Chem.*, **192**, 17 (1951)
87. Horecker, B. L., and Smyrniotis, P. Z., *Federation Proc.*, **10**, 199 (1951)
88. Cohen, S. S., Scott, D. B. M., and Lanning, M. C., *Federation Proc.*, **10**, 173 (1951)
89. Stumpf, P. K., *J. Biol. Chem.*, **182**, 261 (1950)
90. Tewfik, S., and Stumpf, P. K., *J. Biol. Chem.*, **192**, 519 (1951)
91. Tewfik, S., and Stumpf, P. K., *J. Biol. Chem.*, **192**, 527 (1951)
92. James, W. O., and Beevers, H., *New Phytol.*, **49**(3), 353 (1950)
93. Newcomb, E. H. (Unpublished data)
94. Singer, T. P., and Pensky, J., *Arch. Biochem. Biophys.*, **31**, 457 (1951)
95. Stumpf, P. K., *Federation Proc.*, **10**, 256 (1951)
96. Conn, E. E., Vennesland, B., and Kraemer, L. M., *Arch. Biochem.*, **23**, 179 (1949)
97. Vishniac, W., and Ochoa, S., *Nature*, **167**, 768 (1951)
98. Toimach, L. J., *Nature*, **167**, 946 (1951)
99. Arnon, D. I., *Nature*, **167**, 1008 (1951)
100. Hehre, E. J., *Advances in Enzymol.*, **11**, 297 (1951)
101. Peat, S., *Advances in Enzymol.*, **11**, 339 (1951)
102. Hough, L., and Jones, J. K. N., *Nature*, **167**, 180 (1951)
103. *The Enzymes*, **1**, Part 1, 1-530, Part 2, 1-636; **2**, Part 1, 1-790; Part 2 (In press) (Sumner, J. B., and Myrbäck, K., Eds., Academic Press, Inc., New York, N. Y., 1951)
104. *Phosphorus Metabolism*, **1**, 1-774 (McElroy, W. D., and Glass, B., Eds., The Johns Hopkins Press, Baltimore, Maryland, 1951)
105. Cardini, C. C., *Enzymologia*, **15**, 44 (1951)

THE METABOLISM OF PHOSPHORYLATED COMPOUNDS IN PLANTS¹

BY HARRY G. ALBAUM

Department of Biology, Brooklyn College, Brooklyn, New York

This review summarizes existing information concerning the occurrence and metabolism of phosphorylated compounds in plants. While no special review on this subject has appeared before, several articles, one by James (1) and the other by Goddard & Meeuse (2), as well as a book by Bonner (3), have in recent years brought together the important literature in the field. In this article no attempt will be made to catalogue either the older or even the more recent literature. Rather what is planned is, first, a critical examination of the procedures for identifying and estimating phosphorylated compounds in plants, and in this connection to indicate the kinds of compounds which have been found; and second, a discussion of the way in which these compounds participate in certain vital plant activities.

The work with animal tissues and microorganisms in recent years, has focussed attention of the key role of organic phosphorus compounds in a large number of essential reactions. It has been tacitly assumed that these same reactions occur in plants. Indeed, this may be so. However, much of the reasoning in this connection has been done by analogy, and in many cases the essential data are inadequate or lacking entirely. On the other hand, the study and clarification of essential processes which are for the most part peculiar to plants has been made possible with techniques developed for animal tissues. Inversely, it is interesting to point out that some techniques developed in studies with plants have become useful tools for the study of related phenomena in other tissues. It is hoped that the material to be presented in this review will indicate to what extent plant processes involving the participation of phosphorylated intermediates are the same or different from those occurring in animals.

EXPERIMENTAL METHODS APPLICABLE TO THE STUDY OF PHOSPHORYLATED COMPOUNDS IN PLANTS

Much of the experimental data to be described in this first section has been collected in our own laboratory since 1946. Our interests first centered around the demonstration of different phosphorylated compounds, and more recently around the isolation and characterization of adenosine triphosphate from plant sources. During these studies we encountered many procedural problems. In most cases these arose out of attempts to apply techniques devised for animal tissues directly to plant systems. This could not be done, as became presently clear. This first section represents then, in part, a sum-

¹ The survey of the literature pertaining to this review was concluded in October, 1951.

mary of our own experience, which we believe will be useful to others who are working or who plan to work in this field.

In early work on phosphorylated compounds in plants, the tissue was treated with a protein precipitant (e.g., trichloroacetic acid); the phosphorus was then measured in both the acid insoluble residue and in the acid soluble supernatant solution following filtration or centrifugation. The acid insoluble residue was not fractionated further until recently when it was shown to contain phosphorus present in nucleic acids, phospholipid, phosphoprotein, etc. The special difficulties involved in analyzing this residue will be discussed later.

In early studies, the acid soluble fraction was assayed for inorganic phosphorus and organic phosphorus. This information was useful especially in following changes in phosphorus during the development of seeds [Webster, (4), (5); Webster & Dalblom (6)] but gave no information with regard to the specific compounds present. With the discovery of adenosinetriphosphate (ATP) in animal tissues and the demonstration that two-thirds of the phosphorus of this compound is labile (hydrolyzable in 7 min. at 100°C. in *N* hydrochloric acid), labile phosphorus determinations were carried out on acid soluble extracts prepared from plants [Arney (7), James & Arney (8)]. The assumption was made in this work that labile phosphorus is probably synonymous with high energy or ATP phosphorus. Though this is not always true, one still finds references in the physiological literature to this type of analogy.

The first completely up-to-date fractionation procedure for the demonstration of specific phosphorus-containing compounds in acid soluble extracts of tissues was that of LePage & Umbreit (9) in their studies on *Thiobacillus thiooxidans*. LePage & Umbreit were interested in the phosphorus compounds concerned with glycolysis. By using pure compounds and fractionating with barium, these authors were able to show that the following compounds form insoluble barium salts at pH 7: inorganic orthophosphate, adenosinetriphosphate, adenosinediphosphate, phosphoglyceric acid, and fructose diphosphate. The following compounds remained soluble as the barium salts at the same pH: glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, adenylic acid, and diphosphopyridine nucleotide (DPN). They did not imply in their initial publication that these compounds cannot cross-contaminate the different fractions, but they did show that if the amounts of tissue as well as the volumes of reagents were carefully controlled, barium soluble and insoluble compounds did not cross-contaminate to any measurable extent. Briefly, the procedure consisted of deproteinizing with trichloroacetic acid and treating the acid soluble fraction with barium at neutral pH. The barium insoluble precipitate is removed and both the precipitate and the supernatant analyzed for the compounds present. Inorganic orthophosphate, of course, could be determined directly. Phosphoglyceric acid was measured by what appeared to be a specific chemical reaction of Rapport (10). Hexose diphosphate was calculated from fructose (11). The ratio

of ATP to ADP and the specific quantities present were measured by determining total nitrogen as an index of purine present, pentose, and ratio of labile phosphorus to total organic phosphorus present, after deducting that phosphorus resulting from the other compounds detected. The labile phosphorus value, moreover, had to be corrected for the phosphorus hydrolyzed from hexose diphosphate which is considerable (26.5 per cent in 7 min.). For the estimation and identification of the compounds in the barium soluble fraction, the following techniques were employed: Fructose-6-phosphate was again calculated from fructose; glucose-1-phosphate by hydrolysis of the phosphorus which is completely split in 7 min. at 100°C., as well as from its reducing value after removal of phosphorus; adenylic acid from the nitrogen and ribose estimation after correcting for the levels of these present in DPN which, in turn, was estimated from the concentration of nicotinamide. The phosphorus remaining was assumed to result from glucose-6-phosphate; this could be checked independently from its reducing value, making correction, of course, for the reducing values of the other compounds. Results obtained with these techniques in *Thiobacillus* were excellent. Well over 90 per cent of the total acid-soluble phosphorus could be accounted for in terms of the compounds listed above. With slight modification this technique was readily applicable to animal tissues and has been used in studies on shock, cyanide poisoning, and in hormone deficiency.

As is apparent from the procedure described above, the complete identification of the different phosphorylated compounds depends on all the compounds being known. Since the procedure worked so well in animal tissues and bacteria, it did not seem unreasonable to assume that it might work equally well for plants. The first application of this technique to plant tissues was carried out by Albaum & Umbreit (12) on developing oat seedlings. Contrary to expectation, the fractionation procedure turned out to be only partially successful. A few compounds only could be identified with certainty. There was a considerable amount of labile phosphorus but it was already apparent that one could not justifiably equate lability with the presence of high energy phosphorus. As shown above, not only do ATP and ADP have phosphorus which is readily hydrolyzable, but the phosphorus of glucose-1-phosphate is completely labile while the phosphorus of the hexose diphosphate is partially so. Furthermore, on the assumption that the corrected labile phosphorus could be used as an index of ATP and ADP content, the apparent amount of pentose was always in great excess. This could be explained, in part, by the observation that sugar formed from the hydrolysis of starch reacts with the orcinol-pentose-color reagent giving increased density with nonspecific absorption. When large amounts of starch were present, the phosphoglyceric acid reaction instead of yielding a blue color produced one which was almost black due to charring.

When all the possible corrections for known compounds were made, it was apparent that there still was a large amount of phosphorus which could not be accounted for. This led to a search for other types of compounds which

might be present. Indeed, the earlier literature had revealed that there are present in cereal plants large amounts of phytic acid in the form of phytin, and part of the phosphorus which could not be accounted for turned out to be present in this compound. Correcting for phytate still did not give a complete balance indicating the presence of still other phosphorus-containing compounds, not yet identified. The only compounds, therefore, that could be accounted for with certainty in oat seedlings were: inorganic orthophosphate, fructose-6-phosphate, hexose diphosphate, phytic acid, and labile phosphorus which might be associated with ATP. The same kinds of difficulties were encountered by Emerson, Stauffer & Umbreit in their attempts to fractionate the phosphorus-containing compounds in *Chlorella* (13). In this study the only compounds which could be identified with certainty were inorganic phosphorus and hexose diphosphate. Again, evidence was obtained for the presence of ATP, but the expected ratios for nitrogen, pentose, and phosphorus were not realized experimentally.

The occurrence of these abnormal ratios for what might be ATP made it seem desirable to try to isolate this compound from plant tissue. Oat seedlings were selected since a good deal of work had already been done on this material on mechanisms of growth using auxins, and preliminary fractionations for phosphorus containing compounds had been carried out. After some two years of intensive work, Albaum & Ogur (14) reported the isolation from oats of an energy-rich nucleotide in very low yield and of low purity. During these studies, a number of additional difficulties were encountered which bear on the problem of demonstrating these compounds in plants. The purification procedure was patterned on that used for the isolation of ATP from animal tissues. The procedure briefly consists in making a trichloroacetic acid extract of the tissues and fractionating first with barium and then with mercury. ATP, under these conditions, forms an insoluble mercury salt. It very quickly became apparent during the course of this work why so many difficulties had been encountered in the earlier fractionation experiments. The oat plants had so much starch that it was carried along in almost every extract and every precipitate—in spite of the fact that the polysaccharide usually could be removed completely in other tissues by simple treatment of the trichloroacetic acid extract with one volume of alcohol. In addition, it was found that barium phytate was precipitated under almost the identical conditions as ATP. In attempting to substitute spectrophotometric measurement of purine for the analytical determination of purine nitrogen, difficulties were also encountered in the presence of large amounts of materials which absorbed nonspecifically in those regions of the ultraviolet where the absorption of adenine was maximum. In the face of these difficulties, a deliberate search was launched for a new experimental material. This material, we felt, should be low in polysaccharide content, should have little or no phytate, and preferably should not have any components which absorb nonspecifically in the ultraviolet.

The material finally selected for the new isolation experiments from

higher plants was the mung bean (*Phaseolus aureus*). This could be obtained in large quantity in the germinated condition from commercial sources. (It had been previously shown that labile phosphorus, one of the criteria for the presence of ATP, increased during germination of oat seedlings.) Mung beans turned out to have little polysaccharide; free phytate appeared to be lacking; and crude extracts could be obtained with relatively clean absorption spectra so that the adenine could be followed quite readily during purification. From this tissue an ATP of about 70 per cent purity was isolated (15). One of the most interesting features of the isolation procedure was that the nucleotide was purified from a fraction generally discarded in procedures with mammalian muscle (that fraction precipitable as the barium salt at pH 2.0). It behaved differently from animal ATP in some of its reactions and also migrated at a different rate on a paper chromatogram. However, by treatment with mercury, it yielded an ATP which was identical with that found in animal tissues. Identity in this case was established using the criteria of molar ratio of adenine:pentose:labile phosphorus, total phosphorus, rate of color development in the orcinol-pentose reaction (16), and physiological activity. The latter was ascertained by several different enzymatic procedures: measuring the transfer of the terminal phosphorus to glucose in the hexokinase reaction; phosphorylation of pyridoxal to form pyridoxal phosphate; and substitution for animal ATP in the "Cyclophorase" system of Green (17).

It is essential, in demonstrating the presence of ATP- or ADP-like compounds, to have both chemical and physiological data. Recently, Wildman, Campbell & Bonner (18) described a nucleotide associated with a protein from spinach leaves. Because a good portion of the phosphorus was split from the nucleotide in 7 min. in *N* hydrochloric acid at 100°C., the authors assumed that this compound is related to ATP. Indeed, Bonner states elsewhere [(3), p. 210] that "this protein-bound phosphate may play in part the role that ATP plays in other tissues." In this case, the assumption of ATP-like activity is based on the presence of adenine, pentose, and phosphorus, part of which is labile. Pirie (19) has examined a similar cytoplasmic protein from tobacco leaves. He concluded that this protein was a nucleoprotein. If this is true, then the labile phosphorus of Wildman *et al.*, may really be associated with pentose nucleic acid, the phosphorus of which, as is well known, hydrolyzes appreciably in *N* hydrochloric acid. It has not been established that such labile phosphorus is of the high energy type, and until it is convincingly demonstrated that the linkage of phosphorus to C₃ or to C₂ as it occurs in pentose nucleic acid is of the high energy type, this question will remain unanswered.

Additional difficulties in the fractionation procedure described earlier, when applied to green tissue, were demonstrated in some experiments carried out with *Euglena* (20). Labile phosphorus in the tissue extracts was present in great excess of that known to be associated with the nucleotides (in these experiments, adenylic acid, ADP, and ATP were enzymatically determined)

(21). If one relied merely on lability of phosphorus it would appear that *Euglena* contained exceedingly large amounts of an ATP-like material. Further analysis using enzymes, however, revealed that a portion of this highly labile phosphorus resulted from inorganic pyrophosphate. The remainder appeared to be inorganic metaphosphate. The latter was identified through the toluidine blue reaction. In striking a final balance of all of the phosphorus present in this material, it was found that some of the phosphorus ordinarily removed after trichloroacetic acid precipitation could not be accounted for. The only fraction that had not been analyzed for phosphorus was that precipitated with one volume of alcohol and assumed to be polysaccharide. This fraction contained all of the missing phosphorus in the form of inorganic metaphosphate. A study of the early literature reveals that inorganic metaphosphate also occurs in the tissues of higher plants (22). Using a modified fractionation procedure together with specific enzymes, it was therefore possible to demonstrate the presence of the following compounds in *Euglena*: inorganic orthophosphate, pyrophosphate and metaphosphate, adenylic acid, ATP, ADP, DPN, glucose-1-phosphate, fructose-6-phosphate, hexodiphosphate, phosphoglyceric acid, and riboflavin phosphate.

It is important to point out that in the isolation and in the fractionations described above, relatively large amounts of tissue were used. In the case of *Euglena*, cells were harvested from 200 l. of medium; this was equivalent to 24.3 gm. of dried cells. In the isolation of ATP from mung beans, 5 to 10 lb. of plants were used as starting material. In physiological studies it is difficult to work with such large amounts of material and consequently difficult to demonstrate phosphate-containing compounds with any great degree of reliability. One method of approach to this problem was suggested by the work of Calvin and his associates (23) on photosynthesis. This work will be referred to in more detail later. From the viewpoint of methodology, however, they were able to demonstrate the presence of various phosphorylated compounds by a combination of chromatographic and radioautographic techniques. We have applied this method to mung beans using approximately 3 gm. of tissue (24). The seedlings were soaked for short periods of time in solutions containing radioactive orthophosphate. After washing their outer surfaces exhaustively, the seedlings were deproteinized with trichloroacetic acid. Protein-free filtrates after neutralization were chromatographed by either upward or downward migration for periods of 12 to 16 hr. in one or more of the following developers: (a) butanol-acetic acid-water, consisting of 74 ml. *n*-butanol, 19 ml. glacial acetic acid, and 50 ml. distilled water; (b) isoamyl alcohol-malonic acid, consisting of 200 ml. of malonic acid plus 75 ml. of isoamyl alcohol brought to pH 6; (c) ethanol-acetic acid-water, consisting of 80 ml. ethanol and 0.8 ml. acetic acid brought after mixing to pH 3.5 with hydrochloric acid, and with distilled water added to give a final volume of 100 ml. After drying, the paper chromatograms were placed on x-ray film for approximately 48 hr. Using known compounds as

reference standards and determining their position on the paper by enzyme cleavage with alkaline phosphatase and chemical identification of the position of the phosphorus released, the following compounds were demonstrated in mung beans: glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, inorganic orthophosphate, phosphoglyceric acid, phytic acid, and phosphopyruvic acid. The R_f values obtained for these compounds are listed in Table I. The same technique combined with barium fractionation was used by Axelrod, Bandurski & Saltman (25) in pea meal extracts where they demonstrated a similar array of phosphorylated compounds. It should be possible to estimate the actual amount of phosphorus present in these different compounds by extracting the radioactive spots from the paper chromatogram.

TABLE I

R_f VALUES FOR VARIOUS PHOSPHORYLATED COMPOUNDS CHROMATOGRAPHED BY
DOWNWARD MIGRATION IN AN ETHANOL-ACETIC ACID DEVELOPER
FOR 12 HR. IN A CHROMATOCAB*

Compound	R_f value
Hexose diphosphate	0.17
Phosphoglycerate	0.21
Glucose-1-phosphate	0.30
Glucose-6-phosphate	0.40
Inorganic orthophosphate	0.44
Phosphopyruvate	0.62

* University Apparatus Company, Berkeley, California.

The problem of following ATP during growth where only small amounts of plant tissue are available, was solved recently by applying the procedure of Cohn & Carter (26). This involves the use of an anion exchange resin, Dowex #1.² In our work, (27) the following procedure was employed: trichloroacetic acid extracts were prepared from approximately 3 gm. of mung bean seedlings. This extract was precipitated with alcohol and barium at neutral pH after which it was freed of barium with sodium sulfate. It was then made 1 *M* with respect to ammonium hydroxide and poured through the resin several times. Under these conditions, adenosine, adenine, adenylic acid, ADP, a part of the inorganic orthophosphate, and ATP remained on the column. The different compounds were then eluted successively with the following solutions: solution 1 for adenosine 0.01 *M* NH_4Cl in 0.1 *M* NH_4OH ; 2 for adenine 0.01 *M* NH_4Cl in H_2O ; 3 for AMP 0.003 *M* HCl ; 4 for ADP and inorganic PO_4 , 0.02 *M* NaCl in 0.01 *M* HCl ; and 5 for ATP, 0.2 *M* NaCl in 0.01 *M* HCl . In the elution procedure if solution 4, for example,

² Dowex is the trade-mark name for a group of proprietary synthetic ion-exchange resins.

is used first, all the compounds removed by solutions 1, 2, and 3 as well as solution 4, are transferred into the eluate. We were primarily interested in ATP so that we treated the column first with solution 4 and then with 5. Identity with ATP was established by the correspondence between phosphorus and adenine. A typical result from four-day seedlings is shown in Table II. This technique may also be employed with extracts from plants soaked in radioactive phosphorus in which case it becomes possible to measure the specific activity of the phosphorus in ATP. The results obtained with this procedure will be presented later.

TABLE II
ADENINE AND ORGANIC PHOSPHORUS CONTENT OF THREE SUCCESSIVE ELUATES
COLLECTED FROM DOWEX COLUMN TREATED WITH EXTRACT
FROM FOUR-DAY MUNG BEAN SEEDLINGS*

Eluate	μ M adenine	μ M organic P
1	0.67	1.95
2	0.128	0.352
3	0.091	0.219
	0.889	2.515
Molar ratio	0.96	3.00

* After treatment with the crude extract, the column was washed with water, and then with several washes of 0.02 *M* NaCl in 0.01 *M* HCl (solution 4). This was followed by three washes with 0.2 *M* NaCl in 0.01 *M* HCl (solution 5). The analyses presented are for these last washes.

The special difficulties encountered in plant tissues in the quantitative estimation of ribose nucleic acid (RNA) and deoxyribose nucleic acid (DNA) have been pointed out by Ogur *et al.* (28). Identification of RNA in animal tissues has primarily been based on the correspondence between pentose and phosphorus data in the "nucleic acid" extracts obtained by several fractionation procedures (29). Ogur and his associates found that pentosans and polyuronides, general constituents of plant tissues, interfered with the pentose assay for RNA in nucleic acid extracts. In some of the animal work referred to, the nucleic acids were extracted from the cold trichloroacetic acid residues with hot trichloroacetic acid after preliminary removal of phospholipids. Since trichloroacetic acid absorbs strongly and nonspecifically in the ultraviolet, ultraviolet spectra of purines and pyrimidines could not be relied upon without either extensive dilution or removal of the trichloroacetic acid. The use of alcohol as a protein precipitant followed by extraction of the residues with perchloric acid, however, gave fractions which could be assayed in the spectrophotometer. This technique has made it possible to estimate RNA and DNA in a variety of plant tissues including corn roots (28), lily pollen (40),

tobacco leaves (31), mung beans (27), and pea seedlings (31). In this procedure, ribose nucleic acid is removed from the fat-free residues by extended extraction with cold perchloric acid after which the residue is re-extracted with warm perchloric acid. This removes the constituents of desoxyribose nucleic acid. In the case of RNA, identity was based on ultraviolet absorption as well as phosphorus analysis. In the case of DNA, identity was established in terms of ultraviolet absorption, diphenylamine reaction (which measures desoxypentose) and phosphorus. Some minor modifications have been made with different tissues. With mung beans, for instance, it was necessary to extract the phospholipid more exhaustively from the residues than in the case of corn roots before reliable measures of RNA and DNA could be obtained, so that even in plant tissues, it is not always possible to apply a technique without modification when going from one tissue to another.

PHOSPHORYLATED COMPOUNDS IN GLYCOLYSIS

There has now accumulated an impressive mass of evidence to show that phosphorylated compounds in plant tissues play much the same role in glycolysis that they do in animal tissues and in microorganisms. This evidence comes from three sources, the first being the identification of the phosphorylated intermediates involved. The techniques for detecting these intermediates were discussed in the previous section. Table III by way of summary lists the phosphorylated intermediates which have been identified. This list is by no means complete, but it is representative. A second line of evidence is derived from experiments where reactions known to occur during glycolysis also occur in plants. For example, the addition of hexose diphosphate and phosphoglyceric acid to cell-free barley sap leads to the accumulation of pyruvate when precautions are taken for preventing the removal of the latter. This formation of pyruvate can be prevented by fluoride (41). The conversion of phosphoglycerate to pyruvate has also been observed in bean and pea preparations (42). Extracts of many plant tissues enzymatically-split hexose diphosphate to phosphoglyceraldehyde and dihydroxyacetone phosphate (43). Bonner has shown that hexose diphosphate is formed on the addition of glucose to enzyme extracts of *Avena* coleoptiles. The formation of this compound is increased by the addition of ATP. The assumption, of course, is made that ATP initially present was involved when glucose was added alone. Similar data have been obtained by Bonner & Wildman using spinach leaf brei (40). That ATP does indeed occur in plants was shown by Albaum, Ogur & Hirshfeld (15). While all the intermediate steps have not yet been demonstrated, it is clear, since one can establish a chain of evidence starting with glucose and ending with pyruvic acid, that glycolysis does occur and involves the participation of phosphorylated compounds. In plant tissues, pyruvate can then be converted either to alcohol via the intermediate formation of acetaldehyde or in some cases may be converted to lactic acid. The pertinent data are summarized in the recent review of

TABLE III

OCCURRENCE OF GLYCOLYTIC INTERMEDIATES IN PLANTS

Phosphorylated Intermediate	Where Observed	Reference Number
Glucose-1-phosphate	Potatoes	(32)
	Mung bean seedlings	(24)
	Pea meal	(25)
	Oat coleoptiles	(33)
Glucose-6-phosphate	Potato tubers	(34)
	Mung bean seedlings	(24)
Fructose-6-phosphate	Potato tubers	(34)
	Oat seedlings	(12)
	Oat coleoptiles	(33)
	Mung bean seedlings	(24)
	Pea meal	(25)
Fructose 1,6-diphosphate	Barley	(35)
	Oat seedlings	(12)
	Oat coleoptile	(33)
	Pea seed meal	(25, 36)
	Mung bean seedlings	(24)
Triosephosphates (mixed)	Potatoes, pea meal	(37, 38, 39)
Phosphoglycerate	Spinach leaves	(40)
	Barley	(38)
	Mung bean seedlings	(24)
Phosphopyruvate	Barley	(35)
	Pea meal	(25)
	Mung bean seedlings	(24)

Turner (44). It thus appears that the terminal stages in glycolysis in plant tissues may be similar to systems found both in muscle and in yeast.

The operation of the systems described above involves the participation of several coenzymes of which phosphorus forms an integral part. Diphosphopyridine nucleotide (DPN) is involved in the steps between 3-phosphoglyceraldehyde and 3-phosphoglyceric acid; between pyruvic acid and lactic acid in those plants in which this step occurs; and in the case where an alcohol fermentation step exists, between acetaldehyde and ethyl alcohol. In the latter fermentation, thiamine pyrophosphate is also involved. The ubiquitous occurrence of the precursors of these coenzymes in plants (45, 46) argues strongly for their presence. Data on the specific occurrence of the coenzymes of glycolysis, however, are very meager; of these, DPN (47) and thiamine pyrophosphate (cocarboxylase) have been identified in certain plants (48).

The third line of evidence for the presence of a complete glycolytic system is derived from data showing that the individual enzymes are present. This

forms the basis for a separate review paper in this volume and, therefore, will not be discussed at the present time.

The possibility that another mechanism exists in plants for the transformation of some of the phosphorylated intermediates of the glycolytic cycle, especially fructose diphosphate, has been raised in two papers recently published by Tewfik & Stumpf (49, 50). Fructose diphosphate added to extracts of pea seeds (51) yields phosphoglycerate as an intermediate in the pathway to pyruvate. This system is DPN-dependent and is inhibited by low concentrations of iodoacetamide, thus providing evidence for the operation of a triose phosphate dehydrogenase, an essential link in the accepted scheme of glycolysis. However, extracts prepared from pea seedlings (with cotyledons removed), from pea leaves, barley shoots, pumpkin and lupinus seedlings, as well as spinach leaves do not convert added fructose diphosphate to phosphoglycerate. Added phosphoglycerate, on the other hand, is converted by such preparations to pyruvate. The authors conclude from these experiments, after ruling out inhibitors and activators, that in the tissues examined, triosephosphate dehydrogenase is either inactivated or absent, but that the enzymes beyond this point are intact. They also present evidence for a system in these preparations which can oxidize fructose diphosphate through a series of as yet undefined reactions. These require as cofactors flavin adenine dinucleotide (FAD), ATP, and ascorbic acid. Catalase appears to be involved in this system. Phosphoglycerate is not formed as an intermediate. Furthermore, the system is neither inhibited by cyanide, fluoride, or iodoacetamide, indicating that neither cytochrome oxidase, ascorbic acid oxidase, enolase, nor triosephosphate dehydrogenase are involved.

Disregarding for the moment the operation of the aerobic reactions through which fructose diphosphate can be oxidized, the question of the presence or absence of a triosephosphate dehydrogenase in these tissues is still unresolved. It is possible that the enzyme may be present but is inactivated during the preparation of the extracts more readily than it is in seeds. Or to frame the idea otherwise, the enzyme is protected from inactivation by substances present in the seed. In animal tissues, the enzyme is of the SH type, and during preparation as well as during testing precautions must be taken to protect these groups from being oxidized. Recently Racker (52) has found that the isolation of the enzyme from various sources was simplified considerably by the use of Versene, which presumably binds divalent ions which would otherwise rapidly inactivate the enzyme. Tewfik & Stumpf (49), of course, admit the possibility of inactivation.

What appears to be a more serious objection to the extract inactivation hypothesis suggested above comes from the work of Stepka (53), working with intact *Chlorella* cells. When uniformly labeled sucrose or glucose was fed to *Chlorella* cells in the dark, either under aerobic or anaerobic conditions, no significant differences were observed between cells poisoned with 1.5×10^{-4} M iodoacetamide and unpoisoned cells, either in the activity recovered as CO_2 or in the activity incorporated into the insoluble cell material. Further-

more, chromatograms and radioautographs of the soluble fraction from these cells showed no meaningful differences in the metabolic pattern between poisoned and unpoisoned series.

In these experiments there can be no question of enzyme inactivation during preparation, since one is dealing with intact cells. While it is true that the effect of iodoacetate on triosephosphate dehydrogenase was first discovered on intact vertebrate muscle, it is interesting to point out that other intact tissues known to have a complete glycolytic system do not respond similarly. When either 0.001 *M* iodoacetate or 0.005 *M* iodoacetamide was added to manometer vessels containing teased thin strips of scallop muscle (54), no inhibition of glycolysis could be detected; in fact, there was an actual increase in CO₂ production with time. Inhibition could only be produced in this tissue by incubation with these agents prior to carrying out the measurements. In contrast to scallop muscle, the glycolysis of *Thyone* muscle was inhibited by iodoacetate whether it was added before or during measurement. Therefore, it would appear that all intact muscles do not respond in the same way. Whether or not these differences depend on permeability or other phenomena is not clear. Certainly, it is difficult to extend conclusions drawn from intact vertebrate or invertebrate muscle to intact *Chlorella* cells. The question of the presence or absence of this essential enzyme of glycolysis in certain plant tissues will, therefore, have to be kept open. It is particularly important that this question be considered critically in view of the possible participation of this same enzyme in photosynthesis. This will be discussed later.

Currently there is no evidence indicating that a phosphogluconic acid shunt mechanism similar to that described by Cohen (55) in bacteria exists in plants. This mechanism not only accounts for the formation of pentoses but offers an alternative pathway for entering the glycolytic system at the triosephosphate stage. In view of the fact that plants contain large amounts of pentoses and pentosans, some mechanisms for their synthesis must exist. The possibility that pentoses may be built from three carbon fragments, like dihydroxyacetone phosphate, condensing with two carbon fragments is discussed for plants in the recent review by Hassid & Putman (56).

SYNTHESIS OF DI- AND POLYSACCHARIDES

Closely related to the glycolytic system discussed above are those systems which lead to the synthesis of di- and polysaccharides. Generally it is agreed that in animal tissues glycogen synthesis proceeds by an initial esterification of glucose in the presence of ATP to form first glucose-6-phosphate, then glucose-1-phosphate, with glucose-1,6-diphosphate as an intermediate, and finally glycogen through the mediation of a phosphorylase and a branching enzyme. A similar mechanism for the formation of starch seems to operate in some (potatoes, peas) and perhaps in all plant tissues. The presence of a phosphorylase catalyzing the formation of a straight chain amylose, with 1-4 linkages, from glucose-1-phosphate was demonstrated by Hanes in 1940

(32). The mechanism of the synthesis of the branched amylopectin component of starch from the straight chain amylose *in vitro*, however, has been highly controversial. In 1944, Haworth, Peat & Bourne (57) isolated from potatoes a "Q" enzyme which together with the potato phosphorylase catalyzed the synthesis of amylopectin from glucose-1-phosphate. The Q-enzyme, then, performed the same role in amylopectin synthesis as did the branching factor in glycogen synthesis. Since Q-enzyme operated in the absence of inorganic phosphate, it was considered to be of the nonphosphorylative type. Beckmann & Roger (58, 59), however, were not able to duplicate these results. They concluded that the material obtained by the English investigators was not amylopectin, but an artifact which they could duplicate with fatty acids, normally occurring in the material. These formed a fatty acid-amylose complex possessing many properties of amylopectin. The situation was complicated further by a series of papers by Bernfeld & Meutémédian (60, 61, 62, 63), who isolated from potatoes an enzyme which accounted for the formation of the branching in amylopectin. However, this enzyme is of the phosphorylative type. In short, they believed that their enzyme, isophosphorylase, reversibly catalyzed the formation of 1-6 bonds in a reaction analogous to that involving the formation of 1-4 bonds by phosphorylase. The entire question has been re-examined recently by Nussenbaum & Hassid (64). They could find no evidence for the existence of an isophosphorylase, nor were they able to confirm Beckmann & Rogers' claim that synthetic amylopectin is a combination of amylose with fatty acids. They did find, however, that Q-enzyme transformed linear amylose into a synthetic amylopectin whose structure, examined by enzymatic degradation, shows a close resemblance to the structure of natural amylopectins.

The problem of disaccharide synthesis would appear to be intimately related to that of polysaccharide synthesis. Indeed, when a sucrose phosphorylase in bacteria was originally described by Doudoroff and his co-workers (65), it appeared that a similar enzyme in plant tissues might be the solution to the sucrose problem. This enzyme catalyzed the synthesis of sucrose from glucose-1-phosphate and fructose. A sucrose phosphorylase, however, has never been demonstrated in plants. Sucrose, according to the Calvin & Benson group (66), is the first nonphosphorylated sugar formed during photosynthesis. Phosphorylated compounds are probably precursors. Evidence that phosphorus is involved in the synthesis of sucrose comes from a wide variety of experiments, many involving intact plant tissues. Kuranov & Kriukova (67) have shown that leaves from phosphorus-deficient plants lose their ability to form sucrose from hexose. If phosphorus is restored to the medium, this synthetic capacity is regained. Potatoes stored at cold temperatures convert their starch to sucrose. During this process hexose monophosphates and fructose 1,6-diphosphate accumulate. McReady (68) believes that fructose 1,6-diphosphate is probably an essential component for sucrose formation. Because sucrose-producing plants, like beets and peas, contain considerable amounts of hexose phosphates, it has

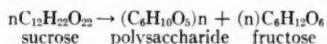
been suggested that sucrose synthesis involves phosphorylations. Support for the phosphorylation hypothesis also stems from the observation that iodoacetate inhibits sucrose synthesis.

Not only does sucrose synthesis in intact tissue appear to require phosphorus, but the process appears to require oxygen. In potatoes, sucrose formation does not occur in the absence of oxygen, nor in the presence of cyanide (68).

Much of the data on the synthesis of sucrose from hexose can be explained if one assumes that phosphorus and oxygen are essential for the generation of ATP bonds which are subsequently utilized to synthesize first glucose-6-phosphate, then glucose-1-phosphate, and finally sucrose through the action of a sucrose phosphorylase. However, as we indicated earlier, such an enzyme has never been demonstrated in higher plants. Another possible approach to this problem comes from the observation reported above that potatoes stored in the cold convert starch to sucrose. In part, this is doubtlessly responsible for the following statement made by James (1):

It has long been known that while the products of starch degradation *in vitro* whether by enzymes or acids are maltose and glucose, the principal product in the living system is sucrose. Thus, although sucrose respiration may occur in leaves and other organs that form no starch, starch respiration does not occur in the absence of sucrose.

Direct sucrose formation from starch has also been reported by Bois & Nadeau (69, 70). If it is assumed that such reactions are reversible, the results would plausibly suggest that the synthesis of starch may proceed through the intermediate formation of sucrose. This would imply that starch and sucrose are interconvertible through exchange and substitution of glycosidic linkages, a phenomenon known to occur in many bacteria. For example, Hehre (71) has recently obtained an enzyme from *Neisseria* which can convert sucrose to amylopectin-like polysaccharide in accordance with the following equation:



Similar enzymes (72) are capable of forming a fructose polymer, levan, from sucrose. Since glucose and fructose polysaccharides occur in many plants, this type of mechanism does not seem unreasonable. It is necessary to postulate that such reactions are reversible and indeed in some bacteria they are (73, 74). The main objection to such a formulation of the sucrose problem, however, lies in the fact that it disregards the well-substantiated chain of reactions described earlier, for the formation of starch from glucose-1-phosphate.

THE METABOLISM OF PHOSPHORUS DURING PHOTOSYNTHESIS

That phosphorus is in some way involved in photosynthesis was suggested by some early work by Emerson, Stauffer & Umbreit (13), who

showed that light caused changes in the distribution of phosphorus containing compounds in *Chlorella*. More recent work by Kandler (75) demonstrated that sudden illumination results in a marked drop of the inorganic phosphorus level, whereas sudden darkening brings about a rise in the inorganic phosphorus level. This author assumed that, with onset of illumination, inorganic phosphorus is esterified to form organic phosphorus.

The recent experiments of the Calvin & Benson group (23, 66) have provided information on the ways in which phosphorus is implicated in this vital plant activity. Coincidentally, their results are most readily interpreted by the existence of a glycolytic system in plant tissues. These workers were studying the photosynthetic dark reactions and they were able to show that in *Scenedesmus* exposure to light for a short period of time led to the reduction of labeled carbon dioxide to yield phosphorylated compounds. Radioactivity in hexosephosphates, triosephosphates, phosphopyruvic acid, and in phosphoglyceric acid were readily demonstrated. In effect, they have detected the synthesis of all phosphorylated intermediates in the glycolytic cycle during photosynthesis. By decreasing the exposure of light, and by analyzing the intermediates which contained labeled isotope, they were able to conclude that the first product formed during photosynthesis was probably 2-phosphoglyceric acid. The presence of the other intermediates meant to them that sucrose and other carbohydrates were synthesized by a reversal of the glycolytic mechanism.

The glycolytic reversal hypothesis for photosynthesis, of course, hinges not only on the demonstration of the phosphorylated intermediates involved, but also on the essential enzymes. And it is here again that the observations of Tewfik & Stumpf on triosephosphate dehydrogenase (48, 49) and the inhibition experiments of Stepka (53), some of which were reported earlier, have further relevance. In another series of experiments, Stepka (53) reasons as follows:

If the path of carbon from CO_2 to sucrose in photosynthesis involves the reduction of 3-phosphoglyceric acid via triosephosphate dehydrogenase, one would expect iodoacetamide poisoned *Chlorella* cells to incorporate less C^{14} into sucrose than the control cells. Secondly, radioactivity should accumulate in 3-phosphoglyceric acid. Neither of these predicted results were observed. Iodoacetamide ($1.5 \times 10^{-4} M$) did not prevent or decrease the amount of activity incorporated into sucrose. Poisoned cells actually incorporated $3.5 \times$ as much radioactive C into sucrose as the controls in one minute periods of photosynthesis with C^{14}O_2 . Furthermore, activity did not pile up in 3-phosphoglyceric acid as expected, but fell along a smooth curve with increased duration of exposure to the poison.

Stepka interprets these observations as indicating a shunt around triosephosphate dehydrogenase. The same objections raised in the section on glycolysis to the use of inhibitors on intact tissues apply here and again serve to focus attention on the crucial nature of the controversy over the presence or absence of the triosephosphate dehydrogenase.

Evidence for an alternative pathway in photosynthesis has recently been

published by Vishniac & Ochoa (76). This pathway involves the participation of phosphorylated coenzymes. It is not altogether proper to term this an alternative pathway, since it might well be one of many mechanisms all of which are operative during photosynthesis. In this work, Vishniac & Ochoa were able to demonstrate the synthesis of malate using chloroplast grana, H_2O , pigeon "malic" enzyme, triphosphopyridine nucleotide (TPN), pyruvate, carbon dioxide, and light. In this reaction light energy leads to a splitting of water with the evolution of oxygen and the production of reducing hydrogens. The latter then reduce TPN. Reduced TPN in the presence of the "malic" enzyme reduces pyruvate to malate with the utilization of one mole of CO_2 . Vishniac & Ochoa effected this reaction with pigeon "malic" enzyme. Tolmach (77) & Arnon (78) have independently studied the same reaction with "malic" enzyme from plant sources. Clearly, this mechanism involves the active participation of TPN which has been found in appreciable quantity in plant tissue (47). If a mechanism existed for the further reduction of malate, e.g., to yield citrate, by a reversal of the Krebs cycle, additional CO_2 could be fixed. This would require the photochemical reduction of other nucleotides, such as DPN, which are involved in this system. This achievement is reported in a more recent publication by Vishniac & Ochoa (79). In addition to the photochemical reduction of TPN which ultimately leads to the formation of malate, these authors also report the reductive carboxylation of α -ketoglutarate to citrate (TPN mediated) and the photochemical reduction of DPN which may then catalyze the following reactions: pyruvate to lactate, the reductive amination of α -ketoglutarate, and the reduction of 1,3-diphosphoglyceric acid to yield hexose diphosphate. The photochemical reduction of DPN is predictably accompanied by the evolution of oxygen. In effect what has been accomplished is an almost complete reversal of the Krebs cycle in the presence of light. This was done by coupling the enzyme systems present in grana with those from animal and bacterial sources. If this kind of cycle operates in photosynthesis within the intact plant, enzymes of the Krebs cycle must be present as well as the essential coenzymes and intermediates.

PHOSPHORYLATION AND THE KREBS CYCLE

Many of the enzymes of the Krebs cycle have been identified in plants. The carboxylating enzymes involved in this cycle and elsewhere in plant metabolism are the subject of a separate article in this volume. Most of the noncarboxylating enzymes, including malic dehydrogenase, isocitric dehydrogenase, aconitase, fumarase, and succinic dehydrogenase, have already been identified at least in some plants. For a recent summary of this work, the reader is referred to the book by Bonner (3). As is clear from the review by Thimann & Bonner (80), almost all the intermediate metabolites have been shown to occur. Of the coenzymes, DPN, TPN, and cocarboxylase have been referred to (47, 48); riboflavin and panthothenate, as indicated earlier, occur so widely in plants that one might expect them to participate in such a cycle if it exists.

In animal tissues the operation of the Krebs cycle is coupled to the esterification of inorganic phosphorus yielding energy-rich bonds usually in the form of ADP and ATP. This esterification appears to take place without the formation, so far as is known, of phosphorylated intermediates in the Krebs cycle, but sometime during the reactions involving the reoxidation of DPN or TPN by molecular oxygen via the cytochrome system. This mode of action is strongly suggested by the experiments of Lehninger (81) using radioactive phosphorus. The esterification of inorganic phosphorus by animal tissues through this combined series of reactions can be uncoupled from oxygen uptake by the use of agents like dinitrophenol, arsenite, gramicidin, and aureomycin.

While there is virtually no literature available on the effect of these agents on the esterification of inorganic phosphorus by plant tissues, there is some indirect evidence that dinitrophenol inhibits several reactions which are probably energy dependent. For example, in carrot tissue (82), dinitrophenol inhibits ion accumulation, while at the same time respiration is unaffected or even increased. This respiratory effect is much like that observed in other tissues. Dinitrophenol also stops protoplasmic streaming (83, 84) and acts as a powerful inhibitor of growth substance action (85). Arsenite, another compound belonging to this category, is also a highly effective growth inhibitor (86). We (27) have measured the effect of arsenite on the uptake, incorporation, and turnover of radiophosphorus in growing mung bean seedlings. Arsenite inhibited all three processes. The primary effect appeared to be on the incorporation and turnover of radiophosphorus in ATP. The specific activity of the ATP was measured by removing it on a Dowex resin, according to the procedure outlined in the section on methods.

An esterification system of the type found in animal tissues involves the participation of terminal oxidases. So far as is known, the only terminal oxidase system which has been shown to be linked to the glycolytic and Krebs cycles through the pyridine nucleotides and which is capable of esterifying phosphorus is the cytochrome system. Cytochrome oxidase and cytochrome have been shown by Meeuse (87) to be present in pea seedlings and appear to be bound to particles which are sedimented at high speeds in the centrifuge, a property which they share in common with mitochondria from animal sources. Indeed, mitochondria from animal sources also are known to contain all of the enzymes of the Krebs cycle. Meeuse's particles contain at least two of these enzymes, succinic and malic dehydrogenases. These data suggest a fundamental similarity between plant and animal systems.

The first clear-cut demonstration that a complete Krebs cycle together with a cytochrome system are present in plant tissues appeared in a recent paper by Millerd *et al.* (88). As the work with pea seedlings had suggested earlier, these enzymes were all associated with cytoplasmic particles which are not distinguishable from mitochondria. These particles, prepared from germinated mung beans and isolated by high speed centrifugation, were approximately 0.5 to 2 microns in diameter and stained with Janus Green B. They were able to oxidize pyruvate completely to CO_2 and H_2O ; this oxida-

tion did not take place unless catalytic amounts of an intermediate of the Krebs cycle were also present, a property characteristic of pyruvate oxidation via the Krebs cycle in animal mitochondria. The terminal oxidase system, as in animals, was of the cytochrome type. Maximum rates of oxidation of pyruvate by these particulates required the addition of inorganic orthophosphate, Mg ions, and either adenylic acid, adenosine diphosphate, or adenosine triphosphate. The fact that adenylic acid could replace ATP, together with the inorganic orthophosphate requirement indicated that oxidation of pyruvate in this case was coupled to the esterification of inorganic phosphate to form energy-rich phosphate in ATP. This, indeed, turned out to be the case. Esterification was detected using radioactive phosphate.

While the only terminal oxidase system in plants which has been shown to function in the above fashion is the cytochrome system, other terminal oxidases are found in the plant kingdom (3) all of which might conceivably operate in this way. However, it should be pointed out that apart from the new system described by Tewfik & Stumpf (50) for the oxidation of fructose diphosphate, in no case has a chain of reactions been established between the carbohydrate substrate and molecular oxygen.

Before terminating the discussion on the esterification of orthophosphate via reduced pyridine nucleotides and the cytochrome system, one other interesting point should be made. As indicated earlier, esterification in this system probably occurs somewhere in the chain of steps between molecular oxygen and the reduced pyridine nucleotides, the levels of which are maintained through the reactions of the Krebs' cycle. There is, however, another mechanism for reducing pyridine nucleotides, and that is through the light reactions in photosynthesis. It is not inconceivable that during photosynthesis the reducing hydrogens formed during the light reaction would reduce all the pyridine nucleotide available, but only part might be used in CO_2 fixation reactions. The remainder might then be reoxidized at the expense of molecular oxygen and lead to the esterification of inorganic orthophosphate with the ultimate accumulation of energy-rich phosphate in the form of ATP. This would provide a mechanism for generating ATP without oxidizing carbohydrate. This rationale is supported by a series of experiments recently carried out by Vishniac (89) who, using a system containing chloroplast grana, liver particles, DPN, ATP, radioactive orthophosphate, and light, was able to show incorporation of the isotope into ATP. This mechanism for the generation of high energy phosphate in the presence of light is quite unlike that proposed by Emerson, Stauffer & Umbreit (13) in their work on *Chlorella*.

THE METABOLISM OF PHOSPHORUS-CONTAINING COMPOUNDS DURING GROWTH

It is generally agreed that one of the chief functions of the systems just discussed is the generation of energy-rich phosphate which is then utilized by organisms for carrying out vital reactions for which energy must be supplied. The phenomenon of growth represents such a complex series of reac-

tions. If growth is dependent on the continuous synthesis of energy-rich phosphate, there must be, first, a system or series of systems which can generate ATP, either by oxidation of carbohydrate or other substrate, or perhaps during the light reaction in photosynthesis as suggested in the last section; and second, there must be a source of inorganic orthophosphate. Seeds, in general, have sufficient phosphorus to meet their early growth demands. As they increase in size, phosphorus must be supplied from exogenous sources. If there is a deficiency of phosphorus, a spectrum of growth aberrations occur. These are too well known to the plant physiologist to require further discussion.

In a monocotyledon like the oat, very little inorganic phosphorus is present in the grain. The concentration of inorganic phosphorus increases rapidly during germination at the expense of a unique phosphorus-containing compound, phytic acid (12), which appears to be the main storage form of phosphorus in all the cereal plants. Phytic acid does not usually occur as the free acid, but as the calcium-magnesium salt (phytin). It has also been found in peanuts, peas, potatoes, and carrots (90). In some seeds it is bound to protein (91). In the mung bean (27) a substance which appears to be identical with phytic acid, on the basis of phosphorus hydrolysis data, is precipitated along with "polysaccharide" with one volume of alcohol from trichloroacetic acid extracts. It is difficult to say whether this material is coprecipitated or is firmly bound. Our inability to detect any free phytic acid in the extracts following "polysaccharide" removal argues against the notion of coprecipitation. In this material, just as in oats, this fraction breaks down during early growth, acting as a source of inorganic phosphorus.

If ATP is related to the growth process, one might expect to observe a correlation between ATP content and extent of growth. This actually occurs in mung beans where it is possible, by using exchange resins to isolate a fraction from small quantities of tissue in which all the phosphorus can be accounted for as ATP (27). In studies with this material, utilizing radioactive phosphorus, it has also been possible to show that the turnover of phosphorus in ATP depends on the seedling content of ATP; i.e., the more ATP present, the more rapidly ATP is built up and broken down. This turnover, as indicated earlier, is inhibited by agents like arsenite which are known to uncouple respiration from esterification.

One other series of experiments carried out in our laboratory (27) bears on the nature of the growth process as conceived of by Thimann and his associates (86, 92). Since 1940, these investigators have developed the thesis that there is a separate respiration associated with growth. This respiration, in oat coleoptiles, for example, is in the neighborhood of 10 per cent of the total oxygen uptake. This means that if one inhibits only 10 per cent of the total respiration with agents like arsenite or iodoacetic acid, growth is completely inhibited. The iodoacetate and arsenite inhibition suggests to Thimann and co-workers that the "growth enzyme" is of the SH type. They believe, furthermore, that growth is mediated through a "4-carbon dicarboxylic acid system" since the inhibition produced by iodoacetic acid can

be "reversed" with succinic, malic, and other 4-carbon dicarboxylic acids. The same type of interpretation has been placed on experiments carried out with pea seedlings where arsenite and other SH inhibitors have been used. Here, too, the effect on oxygen uptake is small although growth may be inhibited markedly.

Our studies carried out with mung beans utilizing radioactive phosphorus (27) have led us to the following conclusions: There is no "growth" enzyme, but rather a series of synthetic reactions involved in growth, all of which require adenosine triphosphate or other energy rich phosphate as an energy source. If the synthesis of ATP is interrupted by agents like arsenite, then some of the synthetic reactions involved in growth are inhibited. Since all of these synthetic reactions are presumably essential, it is expected that inhibiting one or more of these would result in a retardation of growth. The situation is best represented in our opinion by a metabolic pool of ATP which is crucial for all the synthetic reactions involved in growth; this pool is available on a priority basis first to certain reactions, later to others. One should, therefore, expect that some reactions will be inhibited slightly or not at all, others inhibited more, and others almost completely. All, however, are essential to growth. Among the growth changes investigated in our experiments were the syntheses of phospholipid, ribonucleic acid, desoxyribonucleic acid, and adenosine triphosphate.

Since arsenite is an uncoupling agent, there may be little effect on respiration as Thimann observed, but a marked effect on incorporation and turnover of phosphorus into ATP. This we found to be true. The effect of iodoacetate on growth which Thimann recorded can be interpreted as resulting from a block in sugar utilization by inhibition of glycolysis. The "reversal" of this inhibition by 4-carbon dicarboxylic acids probably represents a by-pass around the blocked step. In short all the growth inhibitors observed by Thimann can be interpreted equally well as effects on the maintenance of the ATP pool.

NUCLEIC ACIDS IN PLANTS

A number of recent papers reflect increased interest in nucleic acids in plants. Since the demonstration by Feulgen (93) that nucleic acids of the DNA type were present in plants as well as animal cells, the earlier distinction between "plant nucleic acid" and "animal nucleic acid" was gradually abandoned. Wyatt (94) recently demonstrated that 5-methyl cytosine present as a minor constituent of DNA of various animal cells, is not detectable in DNA isolated from various microorganisms, and is present in significant amounts in DNA from wheat germ. This reopens the question as to whether "plant nucleic acid" may be distinguished by a general compositional difference from "animal" or "microbial" nucleic acid superimposed upon the species differences already reported by Chargaff's group (95). Compositional studies of DNA samples isolated from a variety of plant sources would seem to be in order.

The quantitative variations of DNA level in plant cells have begun to receive experimental attention following the observations of Boivin, Vendrely & Vendrely (96), and Mirsky & Ris (97) that the DNA content of diploid somatic nuclei of certain animal tissues showed species constancy twice that of the haploid sperm. Schrader & Leuchtenberger (98) have examined nuclei of various tissues of *Tradescantia paludosa* by microscopic absorption methods of Feulgen stained preparations. They found different amounts of DNA which did not seem to be attributable to differences in ploidy and suggested different degrees of polyteny in the various tissues. Swift (99) has extended this work on tissues of *Tradescantia paludosa* on tissues of two closely related *Tradescantia* species and on various tissues of *Zea mays*. His observations support the view that small populations of selected (i.e., measurable) nuclei at certain states in the development of cells contain DNA in approximately integral units characteristic of the strain or species. Nuclei with 2, 4, 8, 16, or 32 times the haploid value were observed. Wide variations in interphase cells were consistent with the doubling of DNA during interphase synthesis. In meiosis, DNA was reduced to half the diploid value. If one assumes that the microspore interphase value of Schrader and Leuchtenberger represents a relatively late interphase, all their data with the exception of a high value for meiosis pachytene in the bud anther would seem to fit into the pattern of Smith's expanded study. Ogur *et al.* (30) have reported a study of cellular levels of PNA and DNA during meiosis and mitosis in developing lily pollen using chemical methods referred to in an earlier section of this report. High synchrony of lily pollen during development and division made possible the approach by chemical analysis rather than by spectrophotometric microscopy of individual cells. DNA content dropped sharply in close to predictable fashion at the end of meiosis when four microspores are formed from each microsporocyte. It then increased gradually during the long microspore interphase between meiosis and mitosis. At microspore mitosis the DNA content doubled rapidly with the formation of the binucleate pollen grain. A further gradual increase in DNA content occurred until the opening of the flower. Bryan (100) has recently made observations of DNA content in the development of *Tradescantia* pollen similar to those reported by Ogur *et al.* (30) by microscopic absorption methods. These studies, which show a number of areas of general agreement, still leave several interesting questions which require additional study: Does the selection of small numbers of measurable spherical nuclei in the photometric method tend to exclude values in DNA content intermediate between well defined, integrally related groups? Is pollen microspore interphase typical or atypical of interphase behavior with regard to DNA synthesis? Do generative and tube nuclei contain similar or different levels of DNA and is the level comparable to the haploid or diploid quantity?

LITERATURE CITED

1. James, W. O., *Ann. Rev. Biochem.*, **15**, 417 (1946)
2. Goddard, D. R., and Meeuse, B. J. D., *Ann. Rev. Plant Physiol.*, **1**, 207 (1950)
3. Bonner, J., *Plant Biochemistry* (Academic Press, Inc., New York, N. Y., 537 pp., 1950)
4. Webster, J. E., *J. Agr. Research*, **37**, 123 (1928)
5. Webster, J. E., *Proc. Oklahoma Acad. Sci.*, **9**, 47 (1929)
6. Webster, J. E., and Dalblom, C., *J. Agr. Research*, **41**, 819 (1930)
7. Arney, S. E., *Biochem. J.*, **33**, 1078 (1939)
8. James, W. O., and Arney, S. E., *New Phytologist*, **38**, 340 (1939)
9. LePage, G. A., and Umbreit, W. W., *J. Biol. Chem.*, **147**, 263 (1943)
10. Rapoport, S., *Biochem. Z.*, **289**, 406 (1937)
11. Roe, J. H., *J. Biol. Chem.*, **107**, 15 (1934)
12. Albaum, H. G., and Umbreit, W. W., *Am. J. Botany*, **30**, 553 (1943)
13. Emerson, R. L., Stauffer, J. F., and Umbreit, W. W., *Am. J. Botany*, **31**, 107 (1944)
14. Albaum, H. G., and Ogur, M., *Arch. Biochem.*, **15**, 158 (1947)
15. Albaum, H. G., Ogur, M., and Hirshfeld, A., *Arch. Biochem.*, **27**, 130 (1950)
16. Albaum, H. G., and Umbreit, W. W., *J. Biol. Chem.*, **167**, 369 (1947)
17. Cross, R. J., Taggart, J. V., Covo, G. A., and Green, D. E., *J. Biol. Chem.*, **177**, 655 (1949)
18. Wildman, S. G., Campbell, J. M., and Bonner, J., *J. Biol. Chem.*, **180**, 273 (1949)
19. Pirie, N. W., *Biochem. J.*, **47**, 614 (1950)
20. Albaum, H. G., Schatz, A., Hutner, S. H., and Hirshfeld, A., *Arch. Biochem.*, **29**, 210 (1950)
21. Albaum, H. G., and Lipshitz, R., *Arch. Biochem.*, **27**, 102 (1950)
22. Hardin, E. B., *S. Carolina Exptl. Sta. Bull.*, **8**, 10 (1892)
23. Calvin, M., Bassham, J. A., and Benson, A. A., *Federation Proc.*, **9**, 524 (1950)
24. Albaum, H. G., and Scher, R. (Unpublished experiments)
25. Axelrod, B., Bandurski, R. S., and Saltman, P., *Federation Proc.*, **10**, 158 (1951)
26. Cohn, W. E., and Carter, C. E., *Federation Proc.*, **9**, 161 (1950)
27. Albaum, H. G., *Symposium on Phosphorus Metabolism* (Mich. State Coll. Press, East Lansing, Mich.) (In press)
28. Ogur, M., and Rosen, G., *Arch. Biochem.*, **25**, 262 (1950)
29. Schneider, W. C., *J. Biol. Chem.*, **164**, 747 (1946)
30. Ogur, M., Erickson, R. O., Rosen, G., Sax, K. B., and Holden, C., *Exptl. Cell Research*, **2**, 73 (1951)
31. Goddard, D. R. (Personal communication)
32. Hanes, C. S., *Proc. Roy. Soc. (London)*, [B]**128**, 421 (1940)
33. Bonner, J., *Arch. Biochem.*, **17**, 311 (1948)
34. Arreguin-Lozano, B., and Bonner, J., *Plant Physiol.*, **24**, 720 (1949)
35. Heard, C. R. C., *New Phytologist*, **44**, 184 (1945)
36. Tanko, B., *Biochem. J.*, **30**, 692 (1936)
37. Allen, R. J. L., *Rept. Food Investigation Board*, **196** (1938)
38. James, W. O., Heard, C. R. C., and James, G. M., *New Phytologist*, **43**, 62 (1944)
39. Allen, R. J. L., *New Phytologist*, **39**, 335 (1940)
40. Bonner, J., and Wildman, S. G., *Arch. Biochem.*, **10**, 497 (1946)
41. James, W. O., and Bunting, A. H., *New Phytologist*, **40**, 268 (1941)

42. Neuberger, C., and Kobel, M., *Biochem. Z.*, **272**, 457 (1934)
43. Tewfik, S., and Stumpf, P. K., *Am. J. Botany*, **36**, 567 (1949)
44. Turner, J. S., *Ann. Rev. Plant Physiol.*, **2**, 145 (1951)
45. Withner, C., *Am. J. Botany*, **36**, 355 (1949)
46. Withner, C., *Am. J. Botany*, **36**, 517 (1949)
47. Whatley, F. R., *Enzyme Systems in the Green Leaf* (Doctoral thesis, Cambridge University, England, 1940)
48. Bunting, A. H., and James, W. O., *New Phytologist*, **40**, 262 (1941)
49. Tewfik, S., and Stumpf, P. K., *J. Biol. Chem.*, **192**, 519 (1951)
50. Tewfik, S., and Stumpf, P. K., *J. Biol. Chem.*, **192**, 527 (1951)
51. Stumpf, P. K., *J. Biol. Chem.*, **182**, 261 (1950)
52. Racker, E. (Personal communication)
53. Stepka, W. (Personal communication)
54. Harting, J., *Biol. Bull.*, **93**, 194 (1947)
55. Cohen, S. S., *Bact. Revs.*, **15**, 131 (1951)
56. Hassid, W. Z., and Putman, E. W., *Ann. Rev. Plant Physiol.*, **1**, 109 (1950)
57. Haworth, W. N., Peat, S., and Bourne, E. J., *Nature*, **154**, 236 (1944)
58. Beckmann, C. O., and Roger, M., *Abstracts Am. Chem. Soc., 116th Meeting*, 36 C (New York, N. Y., Sept., 1949)
59. Beckmann, C. O., and Roger, M., *J. Biol. Chem.*, **190**, 467 (1951)
60. Bernfeld, P., and Meutémédian, A., *Nature*, **162**, 297 (1948)
61. Bernfeld, P., and Meutémédian, A., *Helv. Chim. Acta*, **31**, 1724 (1948)
62. Bernfeld, P., and Meutémédian, A., *Nature*, **162**, 618 (1948)
63. Bernfeld, P., and Meutémédian, A., *Helv. Chim. Acta*, **31**, 1735 (1948)
64. Nussenbaum, S., and Hassid, W. Z., *J. Biol. Chem.*, **190**, 673 (1951)
65. Doudoroff, M., Kaplan, N., and Hassid, W. Z., *J. Biol. Chem.*, **148**, 67 (1943)
66. Calvin, M., and Benson, A. A., *Science*, **109**, 140 (1949)
67. Kursanov, A. L., and Kriukova, N. N., *Biokhimiya*, **4**, 229 (1936)
68. McReady, R. M., *Carbohydrate Transformations in Plants with Special Reference to Sucrose Synthesis* (Doctoral thesis, Univ. of Calif., Berkeley, Calif., 1945)
69. Bois, E., and Nadeau, A., *Can. J. Research*, [B] **16**, 114 (1938)
70. Bois, E., and Nadeau, A., *Can. J. Research*, [B] **16**, 121 (1938)
71. Hehre, E. J., *J. Biol. Chem.*, **177**, 267 (1949)
72. Hestrin, S., and Avineri-Shapiro, S., *Biochem. J.*, **38**, 2 (1944)
73. Doudoroff, M., Hassid, W. Z., Putman, E. W., Potter, A. L., and Lederberg, J., *J. Biol. Chem.*, **179**, 921 (1949)
74. Monod, J., and Torriani, A. M., *Compt. rend.*, **227**, 240 (1948)
75. Kandler, O., *Z. Naturforsch.*, **56**, 423 (1950)
76. Vishniac, W., and Ochoa, S., *Nature*, **167**, 768 (1951)
77. Tolmach, L. J., *Nature*, **167**, 946 (1951)
78. Arnon, D. I., *Nature*, **167**, 1008 (1951)
79. Vishniac, W., and Ochoa, S., *J. Biol. Chem.* (In press)
80. Thimann, K. V., and Bonner, W. D., Jr., *Ann. Rev. Plant Physiol.*, **1**, 75 (1950)
81. Lehninger, A. L., *J. Biol. Chem.*, **178**, 625 (1949)
82. Robertson, R. N., *Ann. Rev. Plant Physiol.*, **2**, 1 (1951)
83. Sweeney, B. M., and Thimann, K. V., *J. Gen. Physiol.*, **21**, 439 (1938)
84. Olsen, R. A., and DuBuy, H. G., *Am. J. Botany*, **27**, 392 (1940)
85. Bonner, J., *Am. J. Botany*, **36**, 323 (1949)
86. Thimann, K. V., *Biol. Bull.*, **96**, 296 (1949)

87. Meeuse, B. J. D. (Unpublished data) described in Goddard, D., and Meeuse, B. J. D., *Ann. Rev. Plant Physiol.*, **1** (1950)
88. Millerd, A., Bonner, J., Axelrod, B., and Bandurski, R., *Proc. Nat. Acad. Sci.*, **37**, 855 (1951)
89. Vishniac, W. (Personal communication)
90. McCance, R. A., and Widdowson, E. M., *Biochem. J.*, **29**, 2694 (1935)
91. Fontaine, T. D., Pons, W. A. Jr., and Irving, G. W., Jr., *J. Biol. Chem.*, **114**, 487 (1946)
92. Commoner, B., and Thimann, K. V., *J. Gen. Physiol.*, **24**, 279 (1941)
93. Feulgen, R., Behrens, M., and Mahdihassan, S., *Z. Physiol. Chem.*, **246**, 203 (1937)
94. Wyatt, G. R., *Biochem. J.*, **48**, 584 (1951)
95. Chargaff, E., *Experientia*, **6**, 201 (1950)
96. Boivin, A., Vendrely, R., and Vendrely, C., *Compt. rend. acad. sci., Paris*, **226**, 106 (1948)
97. Mirsky, A. E., and Ris, H., *Nature*, **163**, 666 (1949)
98. Schrader, F., and Leuchtenberger, C., *Proc. Nat. Acad. Sci. U. S.*, **36**, 464 (1948)
99. Swift, H., *Proc. Nat. Acad. Sci. U. S.*, **36**, 643 (1950)
100. Bryan, J. H. D., *Chromosoma* (In press)

STUDIES OF THE PHYSIOLOGY, PHARMACOLOGY, AND BIOCHEMISTRY OF THE AUXINS^{1,2}

BY JAMES BONNER AND ROBERT S. BANDURSKI

*Kerckhoff Biological Laboratories, California Institute of Technology,
Pasadena, California*

INTRODUCTION

Several excellent reviews of auxin matters have appeared during the past few years dealing broadly with the subject as a whole, such as the *Wisconsin Symposium on Plant Growth Substances* which is referred to repeatedly below, as well as reviews of more restricted scope such as those of Thimann (116) and of the previous volumes in this series.

The present review need not, therefore, attempt to cover the entire spectrum of auxin physiology. It will concern a few aspects of the subject only, aspects in which particular progress has been made in recent years. It will attempt to evaluate the work which has been done in each of the several fields, to see in how far an integrated picture can be made of each and to make a few suggestions.

CHEMICAL NATURE OF NATIVE AUXINS

That indoleacetic acid (IAA) is a principal native auxin of higher plants has been evident for some years (25). Not only has IAA been isolated in pure form from plant material [Haagen-Smit *et al.* (61)] but, in addition, the chemical and biological properties of concentrates of the active growth substance from a variety of plant tissues have been found to be similar to or identical with the properties of IAA. The widespread distribution of enzymatic systems for the synthesis of IAA from tryptophan and for the oxidative inactivation of IAA further indicate that this material is of natural occurrence. The principal question regarding the chemical nature of the native auxins at the present time appears to be the quantitative one of exactly what proportion of the total auxin of the plant is indeed IAA. Quantitative methods of auxin identification are needed to resolve this question.

The chemical identification of the native auxins has been difficult and uncertain in the past primarily because the auxin concentration in plant material is very low and a straightforward isolation of the active principle is in general impracticable. For this reason, indirect methods of identification have been used, particularly molecular weight by diffusion and kinetics of destruction in acid and alkaline solution. Both of these methods have now

¹ The preparation of this report was supported in part by the Hermann Frasch Foundation for Agricultural Chemistry.

² The survey of the literature pertaining to this review was concluded in September, 1951.

been shown to be unreliable. Thus, although IAA is the major component of the auxin of the oat coleoptile (141), the molecular weight as obtained by diffusion in crude extracts is too large by a factor of 50 to 100 per cent [Went (138); Wildman & Bonner (141)]. Although pure IAA is rapidly destroyed by heating in 1 *N* HCL in the presence of air, still IAA is protected from destruction in crude extracts or by the absence of oxygen or other oxidizing agents [Holley *et al.* (64)]. The native growth substance of cabbage leaves, now known to be principally IAA (64), is stable to heating in acid in crude extracts of cabbage leaves and for this reason was once held to be an auxin different from IAA [Link *et al.* (81)].

Several new and powerful methods are now available for the chemical characterization of a native plant growth substance even when only small quantities of the material are available. Of these methods, perhaps the most generally useful would appear to be paper chromatography, which might be used both for the isolation and the identification of growth substances. This technique has not yet, however, been applied to the problem on any considerable scale. Further indirect methods for the characterization of IAA are the riboflavin-sensitized photoinactivation of the material, which has been described and applied by Galston (51, 53), and the enzymatic destruction of IAA by IAA oxidase [Tang & Bonner (113, 114); Wagenknecht & Burris (135)]. A spectrophotometric micromethod has been developed (113, 141) which is sensitive to 1 μ g. or less of IAA and which may be used alone or in conjunction with the procedures above. This method, which is based on the Salkowski reaction, depends on the formation by IAA of a red ferric complex in the presence of sulfuric (141) or perchloric (58) acid. It appears to be a valuable tool in the identification and estimation of plant growth substances if suitably and carefully applied. Thus, it may be used for the localization of IAA on chromatographic columns [Linser (82)] and papers [Stehsel (106)] or directly for comparison of the IAA content of a plant extract with the auxin content of the same extract as determined by biological assay [Wildman & Bonner (141)]. Certain materials, including indole, tryptophan, etc., interfere with the determination of IAA if they are present in high concentration. These substances are, however, ordinarily removed by a preliminary concentration of the growth substance fraction to include only ether-soluble organic acids. Certain derivatives of IAA also react with iron to form red complexes in acid solution. Such materials include indole acetamide, esters of IAA such as methylindole acetate, and possibly indole acetaldehyde. It would seem, however, that if these limitations are borne in mind, judicious application of the spectrophotometric assay to the purified organic acid extract of plant material may accomplish the satisfactory estimation of IAA content in many cases [Stehsel & Wildman (107); Teas & Newton (115)].

The chemical nature of the auxin of the oat coleoptile has been studied in some detail in the past and it has been shown that the extractable auxin of this organ is largely or entirely IAA [Wildman & Bonner (141)]. In a new

and extensive study of the subject, Reinert (101) was unable to find any evidence that the coleoptile contains a significant amount of any auxin other than IAA. This conclusion is based on the fact that the coleoptile auxin is completely destroyed by heating in acid, that it is destroyed by the IAA oxidase of pea with kinetics similar to the destruction of pure IAA, and that the activity-concentration relations in the oat curvature test are identical for coleoptile auxin and pure IAA. The major auxin component of the cabbage leaf behaves as IAA in countercurrent distribution [Holley *et al.* (64)] and in chromatography [Linser (82)].

In at least three instances, the growth substance activity of a plant concentrate has been found to reside in part in materials not identical with IAA. In all of these instances, however, the material in question has turned out to be a derivative of or otherwise closely related to IAA. The first of these was the case of the neutral growth substance described by Larsen in 1944 (74). This is indole acetaldehyde and is active only after conversion in the plant to IAA (75). The second case is that of the fruit setting factor contained in immature corn kernels [Redeman *et al.* (98)]. This substance, which is some 100 times more effective than IAA itself, was shown by isolation to be the ethyl ester of IAA (98). Finally, in the countercurrent distribution of the acidic fraction of cabbage leaves, Holley *et al.* (64) found, in addition to IAA, two separable although minor components both active in the Avena curvature test. Although the chemical nature of these components was not resolved, it was shown in a qualitative way that both give the Salkowski reaction and are hence possibly related to IAA in some manner.

The formation of IAA from tryptophan in the higher plant, a reaction first noted by Wildman *et al.* (142) in 1946, involves the production of indole acetaldehyde as an intermediate. The final step in the formation of IAA consists, then, in the oxidation of this aldehyde to the corresponding acid, an oxidation which takes place readily both *in vivo* and *in vitro* with the oat coleoptile (75), leaves [Gordon & Sanchez-Nieva (57)], and roots [Ashby (2)]. Ashby (2) and Larsen (76) have studied the specificity of this conversion and have shown that the enzyme system involved can attack α -naphthylene acetaldehyde, converting it to the corresponding active growth substance, α -naphthylene acetic acid. Similar results have been obtained with 2,4-dichlorophenoxyacetaldehyde which is oxidized to an acidic growth substance, presumably 2,4-D, by extracts of oat coleoptile [Atkinson (3)]. The same interpretation is, no doubt, to be placed upon the work of Bentley (7) in which it is shown that the auxin activity of 2,3,6-trichlorobenzoic acid is equalled by the activity of 2,3,6-trichlorobenzaldehyde.

Although the final production of active growth substance from the corresponding aldehyde is then a relatively nonspecific oxidation, the tryptophan-IAA conversion as a whole shows a greater structural specificity. α -Naphthylalanine, the naphthyl analog of tryptophan, is not, for example, converted to the corresponding growth substance (NAA) in the oat coleoptile (3).

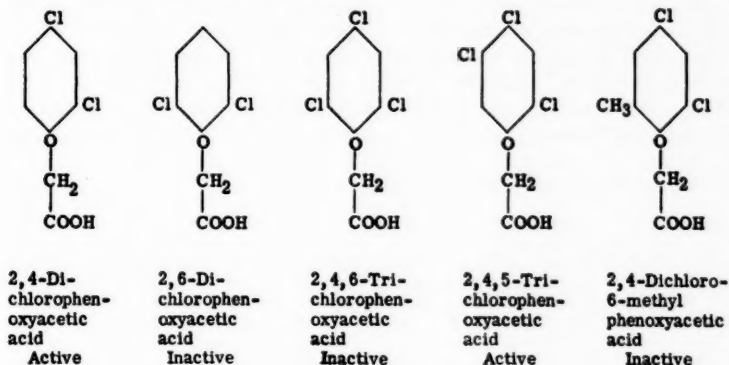
STRUCTURE AND ACTIVITY

An enormous amount of effort has been put into the synthesis and testing of auxin-like compounds and a very considerable number of materials have now been investigated for auxin activity. It is a matter for some concern, however, that the vast amount of information collected has not contributed materially to our understanding of the plant. The empirical rules relating structure to activity (71) have, of course, served to determine fruitful classes of compounds for further study and exceptions to the established rules (117, 139) have served to broaden and better define the concepts involved. Among the compounds synthesized and tested in the course of screening of structures related to the auxins, several compounds of great practical importance have been discovered. No broad new biological concepts have, however, resulted from this screening work nor have we attained any deeper insight into the mechanism of auxin action.

The exciting new attempt of Muir & Hansch (66, 67, 91, 92) to utilize the vast mass of assorted facts concerning structure and activity to deepen our insight into auxin physiology is a particularly welcome one. Their investigations and their suggestions center primarily around a detailed study of the influence of nuclear substitution on the physiological activity of the phenoxyacetic, phenylacetic, and benzoic acids. Promotion of growth in the *Avena* section test (13) provides the basis for their assessment of physiological activity. It is well known that the slight auxin activity of phenoxyacetic acid can be greatly increased by the introduction of halogen substituents into the ring. Thus, *m*- and *p*-chlorophenoxyacetic acid are both active while 2,4-dichlorophenoxyacetic acid (2,4-D) is as active in the *Avena* section test as indoleacetic acid itself. Substitution of the aromatic ring by nucleophilic groups such as methyl does not augment the activity of phenoxyacetic acid as does substitution by the electrophilic halogen group. On the contrary, whereas the introduction of electrophilic groups into the *para* position increases activity, other characteristics of the molecule being suitable, the introduction of nucleophilic groups in the same position tends to decrease activity.

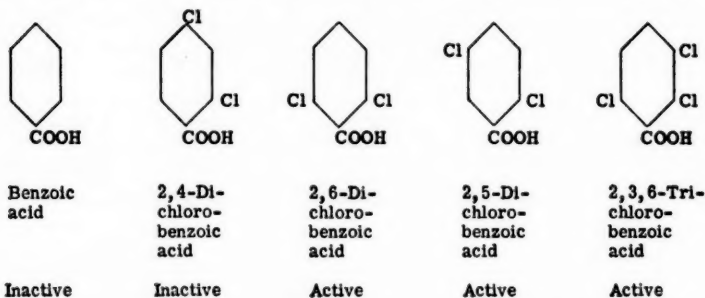
A striking correlation first formulated by Muir, Hansch & Gallup (92) is that substitution of both the 2 and 6 position (both *ortho* positions) of phenoxyacetic acid renders the molecule inactive as a growth substance regardless of the other substituents. Thus, although 2,4-D is active, 2,6-dichlorophenoxyacetic acid and 2,4,6-trichlorophenoxyacetic acid are both inactive. One or both of the *ortho* positions may be blocked by a nucleophilic methyl group rather than by halogen. Thus, 2,4-D can be rendered inactive by introduction of either an electrophilic or a nucleophilic group in position 6. Similar relations hold in the phenylacetic acids in which case, also, activity is dependent upon the presence of an unsubstituted *ortho* position. These observations are in general agreement with such scattered information as is available in the earlier literature (112, 144). That indoleacetic acid itself may require the presence of a free *ortho* group and that these may be the 2 and 4 positions

of the indole nucleus is indicated by the fact that 2,4-di-substituted indole-acetic acids are totally inactive. Substitution in either the 2 or 4 position singly, as for example in the case of 2-methyl- or 2-ethylindole acetic acid, results in greatly reduced activity (66).



On the basis of the observed importance of the *ortho* position to auxin activity, Muir, Hansch & Gallup make the proposal that the basic reaction of an auxin within the cell involves two sites, the carboxyl group and an *ortho* group (92). As a working hypothesis Hansch, Muir & Metznerberg (67) suggest that whereas the carboxyl group may become involved in a peptide or other amide type linkage, the *ortho* position may react with a sulfhydryl group such as the SH group of protein-bound cysteine. The nucleophilic-SH group of cysteine is known from animal experiments to substitute aromatic nuclei with the formation in animals of compounds of the aromatic nucleus-SR type (28). Although this is no more than a suggestion, it does possess attractive possibilities. The auxin mediated reaction is dependent on -SH groups present in the tissue as has been stressed by Thimann & W. D. Bonner (118, 120). The auxin reaction is inhibited by reagents such as iodoacetate, arsenite, and *p*-chloromercuribenzoate (118, 120) or the unsaturated lactones which oxidize, combine with, or otherwise interfere with, -SH groups (133). The general concept of two point attachment of an auxin to its substratum appears also to permit of a more rigorous interpretation of the relation of *cis-trans* and other stereoisomers in relation to physiological activity than has heretofore been possible. The discovery of Bentley [(7); see also (147)] that 2,3,6-trichlorobenzoic acid is active as an auxin, even though both *ortho* positions are substituted, might superficially seem at variance with the concept of Muir & Hansch. In fact, however, the detailed study of the substituted benzoic acids has made possible a searching test of the concept of the *ortho* position as an essential functional group of the auxin molecule. The rules which govern activity in the substituted benzoic acid series are different from the rules which apply to the phenoxyacetic and

phenylacetic derivatives. Thus 2,4-dichlorobenzoic acid is totally inactive while the 2,6-dichloro compound is active (66, 137). 2-Chlorobenzoic acid is very slightly active, but this activity is increased by a second electrophilic group in either the *meta* or *ortho* position, in contrast to the phenoxy series where *para* substitution gives the greatest augmentation of activity. In summary, benzoic acids are inactive unless one or both *ortho* positions are substituted by an electronegative group such as halogen. This provides strong support for the view of Muir & Hansch that substitution at the *ortho* position is involved in the auxin reaction, for in the benzoic acids the tendency to undergo *ortho* substitution is increased when electronegative



groups are originally present in the same position. This is to be contrasted to the state of affairs in the phenoxyacetic acids in which the tendency to undergo *ortho* substitution is decreased by the presence of electronegative groups at this position. The differences in reactivity of these two series of compounds lie in the proximity of the *ortho* position to the electron-attracting carboxyl group in the case of the benzoic acids, as contrasted to the proximity of the *ortho* position to the electron-rich phenoxy-linkage in phenoxyacetic acids. Our present body of empirical information concerning substitution and activity in the phenoxyacetic, phenylacetic, naphthalene acetic, naphthoxyacetic, and benzoic acids apparently can, in fact, be interpreted coherently in terms of a requirement for a critical electron density at those positions of the aromatic nucleus which are *ortho* to the side chain.

That the *ortho* position of the growth substance molecule does actually react with an appropriate nucleophilic group *in vivo* is indicated by the fact that during the course of the growth of *Avena* coleoptile sections in solutions of 2,6-dichlorobenzoic acid, inorganic chloride is liberated (67). Thus, in 8 hr. some 28 per cent of the chloride of the growth substance appeared as free chloride (67). This amount was greater than appeared when sections were incubated in an equal amount of the more readily hydrolyzed but physiologically inactive 2,4-dichlorobenzoic acid. Further study of this phenomenon should make it possible to identify the exact nature of the reactant involved and perhaps to discover the way in which auxins are bound in the plant.

These investigations, then, point strongly to the *ortho* position as an important reactive site of the growth substance molecule. They suggest that auxins may react with their substrate or carrier molecules at two points, namely, the *ortho* position as well as the carboxyl group. The concept of the *ortho* position as a reactive site of the auxin molecule opens new approaches to the study of the native substrate of the growth substances.

AUXIN ANTAGONISTS AND THE ANTIAUXIN CONCEPT

Certain compounds, which are inactive or essentially so as auxins, are nonetheless physiologically effective and elicit plant responses which result from inhibition of the action of auxin within the plant. Such antiauxin activity may be demonstrated in one of two general ways. In the first place, the growth or other responses induced by the application of auxin to a tissue low in native auxin may be shown to be depressed in the presence of non-toxic concentrations of the auxin antagonist. In the second place, the auxin antagonist may be applied to tissues or intact plants, which contain abundant native auxin. In this case, symptoms interpretable in terms of auxin deficiency may appear as, for example, lessened growth of the internodes and diminution of apical dominance. These criteria are not of themselves sufficient, of course, to establish a compound as an auxin antagonist. The crucial point is that the growth inhibition or other symptom induced be alleviated or abolished by the addition of further auxin.

The concept that the metabolism of a substrate may be blocked by a molecule of related structure, capable of reaction with the appropriate enzyme but incapable of metabolic transformation, was clearly recognized by Quastel (97) for the case of the inhibition of succinate oxidation by malonate. We say that malonate is a competitive inhibitor of the enzymatic oxidation of succinate. An auxin antagonist, a competitive inhibitor of the action of auxin, should, therefore, follow the general rules of competitive inhibition. Of these, the most important is that the extent of inhibition depends not on the absolute concentration of inhibitor but on the mole ratio between inhibitor and substrate in reaction mixture. The general principles which should govern growth inhibition by auxin antagonists are clearly developed in the early paper of Skoog, Schneider, & Malan (103) which is concerned with γ -phenylbutyric acid and its interaction with indoleacetic acid (IAA) in the *Avena* test. γ -Phenylbutyric acid possesses a very slight activity in the *Avena* test (roughly 100,000 times less than that of IAA). The data of Figure 1 show that it is, however, active in inhibiting IAA induced curvature in the *Avena* test. For any given concentration of γ -phenylbutyric acid, this inhibition is large at low auxin concentrations, small at high auxin concentrations. *Avena* coleoptile growth may, of course, be inhibited by other types of inhibitors. Figure 1 includes data on a typical inhibitor whose action is not related directly to auxin, namely, coumarin (132). Inhibition by a given concentration of this substance, an unsaturated lactone, is not decreased as auxin concentration is raised (19). Inhibition by

by the unsaturated lactones involves the destruction of essential SH groups within the cell and is more or less competitive with SH-protecting substances such as BAL (2,3-dimercaptopropanol; Dimercaprol) [Thimann & Bonner (121)].

It is proposed, then, that the term auxin antagonist be reserved for those materials whose effectiveness as auxin inhibitors depends on the inhibitor/auxin ratio. This use of the term would be consistent with the use generally applied in relation to metabolite antagonists. A further qualification should perhaps also be made, namely, that an auxin antagonist is a compound related in chemical structure to the auxins but possessed of the above-outlined

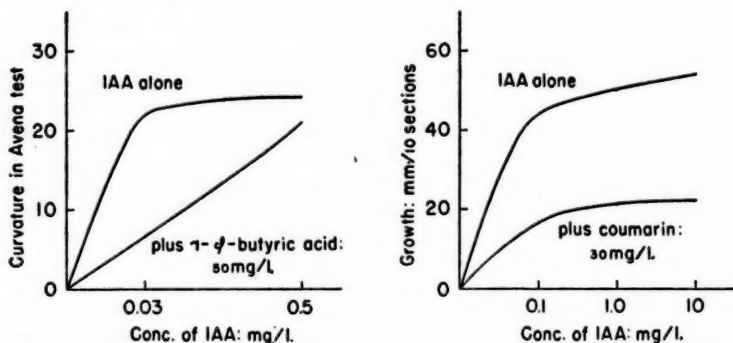


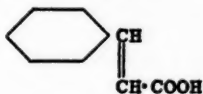
FIG. 1. Inhibition of auxin-induced growth responses by an auxin antagonist (left) and by a nonauxin related inhibitor (right). Left: Inhibition of Avena curvature test by γ -phenylbutyric acid, after Skoog *et al.* (103). Right: Inhibition of growth of Avena sections by coumarin, after Bonner (19).

physiological properties. With this qualification, the term auxin antagonist would appear today to comprise only compounds which are relatively specific to the inhibition of auxin activity and the auxin antagonist concept to be an experimentally useful one.

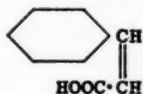
Demonstration that a particular substance acts as a competitor or antagonist to an auxin can be put on a more rigorous basis than is possible from the qualitative considerations outlined above through use of the kinetic analyses of Lineweaver & Burk (80), Goldstein (55), and Strauss & Goldstein (105). Treatment of the Avena test data of Skoog *et al.* (103) by these methods indicates that γ -phenylbutyric acid is a true competitive inhibitor of IAA in the Avena test.

The most thorough analysis to date of the action of an auxin antagonist is that of Van Overbeek, Blondeau & Horne (130) which deals with *trans*-cinnamic acid. *Trans*-cinnamic acid is totally inactive as an auxin in contrast to the related *cis*-cinnamic acid which is active although less so than indoleacetic acid itself. *Trans*-cinnamic acid is active, however, in decreasing

the growth response brought about by *cis*-cinnamic acid in the pea curvature or pea section test. The inhibition elicited depends not on the concentration



Trans-cinnamic acid



Cis-cinnamic acid

of inhibitor in the reaction mixture but on the ratio of inhibitor to growth substance. Thus, the addition of *trans*-cinnamic acid in a molecular ratio of 100 *trans* to 1 *cis* molecule brought about a 50 per cent reduction of the growth elicited by *cis*-cinnamic acid. Viewed in another way, the relative inhibitory effect of a given concentration of *trans*-cinnamic acid decreased as the concentration of *cis*-cinnamic acid in the reaction mixture increased.

Not only is *trans*-cinnamic acid active in antagonizing the growth effects of *cis*-cinnamic acid, but it is also active competitively against indoleacetic acid, naphthalene acetic acid, and 2,4-D. Approximately 1000 molecules of *trans*-cinnamic to one of active growth substance are, however, required to cause 50 per cent inhibition of an IAA-induced pea test curvature. Somewhat different mole ratios appear to be required for inhibition of the action of IAA, of naphthaleneacetic acid, and of 2,4-D in the pea section test. Of the auxin antagonists studied to date only *trans*-cinnamic acid is known with certainty as a natural product and in fact as a naturally occurring plant growth inhibitor [Bonner & Galston (21)].

A second compound which possesses the ability to antagonize the growth promoting effects of auxin is 2,4-dichloranisole [Bonner (17)], a substance related to 2,4-D but lacking the terminal carboxyl group of the acetic acid side chain. Like inhibition by *trans*-cinnamic acid, dichloranisole inhibition depends on the molecular ratio between inhibitor and auxin. The ratio required for 50 per cent inhibition of *Avena* section growth (19) with 2,4-D as the auxin is approximately 20 molecules of inhibitor to 1 of auxin. Thus, dichloranisole (DCA) appears to be much more effective than *trans*-cinnamic acid as an auxin antagonist. Its slight solubility in water limits the concentration range over which it can be used. In addition, dichloranisole appears to act competitively only at low auxin levels and exerts a considerable nonspecific inhibition at higher auxin levels (19). These facts appear to exclude it from consideration as a general experimental tool.

Attention was first called to 2,3,5-triodobenzoic acid (TIBA) because of the morphogenetic effects which this material exerts on developing shoots (145). Galston (50) next noted that TIBA suppresses the activity of IAA in the *Avena* curvature test, and, in addition, inhibits both internode elongation and apical dominance in soybean. Unlike *trans*-cinnamic acid and DCA, TIBA itself possesses some slight growth activity (91, 119). That it is nevertheless effective in inhibiting the action of IAA in the *Avena*

test has, however, been confirmed by several investigators (91, 119), the molecular ratio of inhibitor to auxin needed for 50 per cent inhibition varying from 8 to 34 in the several cases. Inhibitions caused by TIBA in the *Avena* or other growth tests have never been found to be fully reversible by added auxin and, in fact, according to de Waard & Florshütz (44), if TIBA is added to the tissue 1 hr. before the application of auxin, the resulting inhibition is not reversible at all. This may indicate that in TIBA we are dealing with an antagonist which specifically but irreversibly combines with or otherwise destroys those sites in the cell which are essential to auxin action.

The action of γ -phenylbutyric acid, unlike that of TIBA, would appear to be wholly competitive, at least in the *Avena* test. The mole ratio of inhibitor to IAA needed for 50 per cent inhibition in the experiments of Skoog *et al.* (103) varies roughly between 350 and 600. It is of interest to note that γ -phenylbutyric acid, like TIBA, is slightly active as an auxin in the *Avena* section test but, like TIBA, is an antagonist of other more active auxins. As Hitchcock & Zimmerman (69) have so aptly expressed it in another connection, "In view of the remarkable growth regulating properties of TIBA . . . , the question whether these regulators exhibit cell elongating activity in the *Avena* test is of minor importance. . . ."

The relations of roots to auxin are somewhat different in general outline from those of stems and of *Avena* coleoptiles. Thus, roots are, by and large, inhibited in their growth by added auxin, although very low concentrations (in general, 10^{-8} M or less) may slightly increase root growth. Burström (29) has suggested in this connection that two histologically different and separable reactions may be involved in the responses of roots to applied auxin. The first of these, according to Burström, is the time interval between cell division in the meristem and initiation of cell elongation, an interval which Burström suggests may be shortened by low concentrations of auxin. The second auxin sensitive process in the root, according to Burström, is the rate of cell elongation, which appears to be decreased by added auxin. It is this latter process which is ordinarily dominant in studies of the effect of auxin on root growth. In any case, roots are inhibited in their growth by auxin concentrations above approximately 10^{-8} M. Burström has now shown that certain substances related in structure to the auxins are not only ineffective in inhibiting root growth but actually increase root growth by a factor of two or more. This action may be reversed by auxin, the extent of the reversal depending on the auxin concentration. Of the materials discovered by Burström as active in increasing root elongation, the most interesting are auxin derivatives in which the acetic acid side chain is replaced by an isobutyric group. Thus, 4-chlorophenoxyisobutyric acid (29) and indole-3-isobutyric acid (31) are both effective in increasing root growth when supplied at concentrations of 10^{-6} to 10^{-8} M. The mole ratio of 4-chlorophenoxyisobutyric acid to IAA needed for 50 per cent reduction of the growth-stimulating effect is approximately 350. Although the 4-chloro-substituted compound appears to be the most active of the phenoxyisobutyric acids

tested by Burström, a variety of differently substituted compounds of this group are also more or less effective. Phenoxyisobutyric acid itself is active in increasing root growth as are its 2- and 3-monochloro substituted derivatives (30). 2,4-Dichloro, 2,4,5- and 2,4,6-trichloro, as well as 2,3,4,5,6-pentachlorophenoxyisobutyric acid are all active. Thus, the *ortho* positions may both be blocked and the compound yet retain auxin antagonist activity.

The substituted phenoxyisobutyric acids are not only effective in increasing root growth, but also bring about other morphogenetic responses which suggest that they may function broadly as auxin antagonists (83). That effectiveness in increasing root growth is a good criterion for the screening of potential auxin antagonists is suggested not only from Burström's work, but also by the fact that during the course of an earlier search for an auxin antagonist (17) the substance 2,4-dichloroanisole was selected for trial because of its reported activity in increasing the growth of corn roots (123). A variety of further substances related to the auxins in structure have been reported to be effective in increasing root growth and have been included in Table I, which presents a summary of information thus far available on materials which appear to possess auxin antagonist activity.

What can we now conclude as to the alterations in chemical structure which may transform a compound from an active auxin to an antiauxin? The following tentative proposals may be made on the basis of the information at hand:

(a) Molecules which are themselves weakly active as auxins may act antagonistically to molecules of more active compounds (γ -phenylbutyric acid, possibly TIBA).

(b) Certain specific alterations of the side chain of an active compound may transform it into an antagonist. Such alterations include, for example, substitution of the isobutyric for the acetic side chain, elimination of the carboxyl group from the side chain (as in 2,4-dichloroanisole), or isomerization (as in the case of *trans*-cinnamic acid). In so far as we can now judge, nuclear substitution and even the exact nature of the ring system involved is less important in determining auxin antagonist activity than it is in determination of auxin activity itself.

It is of interest to note that in several instances alteration of the active molecule by a single structural or configurational step may result not only in loss of auxin activity but in transformation of the molecule into an antiauxin. Thus, indole- α -propionic acid is active (one optical isomer only) but introduction of two methyl groups at the *alpha* position results in a compound with antiauxin activity. It would quite evidently be of interest to examine for antiauxin activity other groups of auxin analogs which have been reported to be inactive as auxins.

Of what interest can the general subject of the antiauxins be to plant physiology and to agriculture? These materials hold promise both as research tools and as crop control agents. From the standpoint of auxin lore, the modest amount of work done with the auxin antagonists has already shown us that in general an antagonist to one active growth substance is an antago-

TABLE I

A SUMMARY OF INVESTIGATIONS OF POSSIBLE AUXIN ANTAGONISTS

Substance	Antagonistic to:	Approx. mole ratio for 50% inhibition	Test	Extent of reversal by auxin	Author
1. λ -Phenylbutyric acid	IAA	350	Avena test	much	Skoog <i>et al.</i> (103)
2. Trans-cinnamic acid	<i>cis</i> -cinnamic	100*	pea section	much	Van Overbeek <i>et al.</i> (130)
	IAA	10	pea section	much	
	2,4-D	10,000	pea section	much	
	NAA	1,000	pea section	much	
3. 2,4-Dichloroanisole	2,4-D	20	Avena section	much	Bonner (17, 19)
	IAA				
4. 2,3,5-Triodobenzoic acid	IAA	8	Avena test	—	Galston (50)
	IAA	26	Avena test	little	Thimann & Bonner (119)
	IAA	34	Avena test	little	de Waard & Florschütz (44)
5. <i>p</i> -Chlorophenoxy isobutyric acid	IAA	300	wheat root	much	Burström (29)
6. Indole-3-isobutyric acid	native root auxin	approximately as 5	wheat root	—	Burström (31)
7. 2,4-Dichlorophenyl sulfone acetic acid	native root auxin	less active than 5	wheat root	—	Wilske & Burström (143)
8. (1 Naphthyl-methyl sulfide) propionic acid	2,4-D	100	flax root	complete	Aberg (1)
	NAA			complete	
	IAA	100		much	

* Computed on basis of undissociated molecules only.

nist to many, perhaps to all. This would appear to strengthen the view that the action of such different types of substances as IAA and 2,4,-D is basically similar. From the research standpoint also, the use of auxin antagonists may permit us to lower artificially the level of effective auxin in an otherwise normal plant and thus to identify more clearly the nature and extent of the processes which are auxin-controlled in the plant. Knowledge of the nature of action of antiauxins may well increase our understanding of the normal auxin economy of the plant. It becomes even more apparent that the effective auxin level in a living tissue is a resultant not only of the concentration of auxin itself in the tissue but also of a variety of naturally occurring inhibitors of auxin action. Some of these inhibitors are doubtless coumarins or coumarin derivatives; others may be actual auxin antagonists. From the standpoint of crop control, the development of an understanding of auxin antagonists and their use would appear to be a reasonable approach to the problems which are the converse of those which we now control by the application of

auxins. Thus, when we want to make the fruit stay on the tree we apply an auxin. To make it drop off we might try an auxin antagonist.

AUXINS AND FLOWERING

It is now clear that flower initiation may be suppressed in several species by the application of auxins and that, contrariwise, flower initiation may be promoted under certain circumstances by the application of auxin antagonists. Our knowledge of the auxin relations of the flowering process is confined principally to results obtained from artificial application of active substances. It would appear to be quite possible, however, that flowering may normally be influenced or even controlled by fluctuations in the auxin economy of the plant.

A great number of plants of a great number of species have in the past been sprayed or otherwise treated with a wide variety of active growth substances. In only a small number of cases have any dramatic effects of auxins on flower initiation been recorded, and only in a still smaller number of instances has it been clearly shown that floral initiation has been suppressed by auxin application. This anomaly appears to result from at least two conditions which circumscribe the detection of auxin effects on flowering. In the first place, plants differ greatly in the ease with which flowering is inhibited by auxin application. In the second place, the inhibition of flowering appears to be best achieved by continued application of concentrations of growth substance well below those which are ordinarily given in experiments designed to influence growth in general and in which one or a few applications at most are made. Thus, in the light of our present knowledge, it is no occasion for surprise that effects on flowering have been observed in so few of the past experiments on auxin application to intact plants.

The first specific mention of the inhibition of floral initiation by applied auxin was by Dostal & Hosek in 1937 who applied indoleacetic acid in lanolin paste to the leaves of ripe-to-flower shoots of *Circaea* (45). A similar clear inhibition of floral initiation by IAA, applied in lanolin paste, was achieved in 1938 by Hamner & Bonner with *Xanthium*, a photoperiodically sensitive short-day plant (65). That flowering of the pineapple may be inhibited by applied growth substance was demonstrated by Cooper in 1942 (41). Chododny in 1939 (35) and Galston in 1943 (48) both clearly formulated the view that high auxin levels in the plant may be unfavorable to flowering. Despite these early indications of a relation between auxin and flowering, interest in the subject did not become general until 1947, in which year the first systematic report of the inhibition of photoperiodic induction of *Xanthium* by auxin appeared (124). Indoleacetic acid, naphthalene acetic acid, and 2,4-D were all found to be effective in relatively low concentration if continuously applied (24, 124). That the inhibitory effect of auxin on flowering is exerted in the leaf was also indicated. Similar relations obtain in the inhibition of floral differentiation of the short-day plant *Kalanchoe* [Harder & van Senden (68)]. Entirely similar, if less spectacular, effects of auxin in inhibition of flowering have been found for short-day teosinte [Leopold & Thimann (77)].

Inhibition of flower formation in further short-day plants by periodic spraying with IAA or NAA has been reported by von Denffer & Gründler (134), by Liverman (83), and by Lora (84). The inhibition of flowering of short-day plants by applied auxin occurs only when the auxin is supplied during the photoperiodic induction period and is ineffective if supplied immediately after the expiration of this period (79, 83). Auxin treatment not only prevents the expression of flowering in the treated plant, but it also prevents the synthesis in or export from the leaves of any flower-forming stimulus, since plants treated with auxin during the induction period are unable to induce flowering in untreated graft partners (24, 124). Inhibition of floral initiation does not result simply from a general growth inhibition, since plants in which flowering is suppressed by auxin treatment continue normal vegetative development (24, 68).

The effects of auxin treatment on the flowering response of photoperiodically sensitive long-day plants are still somewhat obscure. Complete suppression of flowering with continued vegetative growth results from repeated auxin (daily spray) treatment of *Calendula* [von Denffer & Gründler (134)]. In three further long-day species, flowering was merely retarded by IAA application, the retardation being largely unspecific in nature and consisting of a general delay in development. With Wintex barley, on the other hand, continuous application of low concentrations of auxin (IAA or NAA) may actually increase the number of floral primordia found [Leopold & Thimann (77)].

We have little information on the influence of repeated or continuous auxin treatment on flowering of photoperiodically indeterminate plants. The data available suggest, however, that in this group, auxins so applied tend to delay or inhibit flowering. Thus, Laibach & Kribben (73) achieved suppression of flowering of axillary shoots of *Cucumis* by application of NAA or IAA (in paste form) and vegetative growth continued in the shoots where flowering was suppressed. IAA applied to the root system delays flower initiation in petunia and soy bean [Green & Fuller (60)]. Many of the reports concerning the effects of growth substance treatment on reproductive behavior of indeterminate plants have, however, been uncritical in that the appearance of flower buds rather than of floral primordia has been used as the criterion of reproductive response.

In summary, application of auxin retards or completely suppresses floral initiation in several short-day plants. The effect is exerted during the photo-inductive period and appears to be exerted upon the leaves. Striking qualitative effects of auxin application to long-day and indeterminate plants have thus far been reported in only a few scattered cases. The responses which have been recorded include both the inhibition and the promotion of flowering. With short-day as well as with certain long-day and indeterminate plants, inhibition of floral initiation by auxin can be achieved in such a manner as to result in continued vegetative growth.

Just as floral initiation may be inhibited by applied auxin, floral initiation

may be promoted by applied antiauxins, i.e. auxin antagonists. Perhaps the clearest instance of this relation is found in *Xanthium*. The cocklebur flowers only on a regime of short days and long nights. If the night is interrupted either by a light flash or by low-intensity light of longer duration, the plants remain vegetative (65). The application to *Xanthium* of a variety of auxin antagonists causes the plant to flower even when the photoperiodic regime is unsuitable so that control untreated plants remain vegetative. Thus, TIBA and DCA can overcome the effect of low-intensity supplementary light [Bonner (18)]. TIBA and a variety of other auxin antagonists are able to overcome the inhibitory effect of a light flash given in the center of a long, dark period (83). The effect of applied antiauxins in induction of flowering have thus far been achieved, however, only under photoperiodic conditions near the critical lengths (18, 79, 83). The effects of TIBA and DCA in inducing flowering in *Xanthium* are not specific for these two compounds but extend also to the substituted isobutyric acids which, as shown above, also possess auxin antagonistic activity. Thus, Liverman (83) has found 2,4-dichlorophenoxyisobutyric acid to be an effective agent for floral initiation in *Xanthium*.

It may be noted that the effect of auxin antagonists in inducing flowering in *Xanthium* is inhibited by applied auxin just as is normal flowering (18). In a complementary fashion, the effect of auxin in inhibiting normal flowering may be suppressed by applied auxin antagonists such as TIBA or DCA [Bonner & Thurlow (24)].

The relation of TIBA to floral initiation was originally discovered by Zimmerman & Hitchcock (145) on a photoperiodically indeterminate plant, the tomato. Application of TIBA resulted in the formation of an increased number of flower primordia per cluster, in the induction of terminal flower buds, and, most spectacularly, in the induction of flowering of seedlings. Thus, de Waard & Roodenburg have shown that in TIBA-treated plants, flower buds may appear after the formation of only 3 leaves instead of after 15 to 19 leaves as is normal for the variety which they used (43). The early findings of Zimmerman & Hitchcock with the tomato have been confirmed and extended not only by de Waard & Roodenburg but also by Gorter (59), Zimmerman & Hitchcock (146), Laibach & Kribben (73), Osborne & Wain (95), and others. Osborne & Wain have also reported that a second substance, α -(2-naphthoxy) phenylacetic acid, has an effect on tomato similar to, although less striking than, that exerted by TIBA.

Quantitative effects of TIBA in increasing the numbers of floral primordia which develop on photoperiodically short-day species grown on short days have been noted for soybean by Galston (48, 49), for *Xanthium* [Livermann (83)], and for *Kalanchoe* [Esteves-de-Sousa (46)] in plants given a minimum induction period. In general, however, application of TIBA to photoperiodically indeterminate species other than tomato and *Cucumis* have met with negative results [Snyder (104); Tumanov & Lizandr (125); Whiting & Murray (140)]. It would seem reasonable, however, to look into the possibili-

ties with the indeterminate plants of simulating in some manner the threshold flowering conditions which must obtain with short-day plants before clear flower-inducing effects of TIBA can be demonstrated.

In summary, photoperiodic induction can be inhibited in short-day plants under suitable conditions by application of IAA, NAA, 2,4-D, and other auxins. Flower initiation can be brought about under threshold conditions by a number of substances including TIBA and other auxin antagonists. These relations suggest an involvement of auxin with the flowering of short-day plants. Information on indeterminate plants, while scattered and incomplete, does, in general, indicate that in these groups also auxins may inhibit flowering and auxin antagonists promote flowering.

This brief review has concerned itself wholly with the auxin relations of flowering as studied by the treatment of the plant with exogenous growth substances. It will be clear that a final and definitive understanding of the part played by auxin in flowering cannot be attained without knowledge of the changes in auxin level which take place in the plant during, for example, photoperiodic induction. It is hard, however, to get satisfactory and convincing information about auxin levels in leaves of photoperiodically sensitive plants because of the presence of inhibitors of the *Avena* tests in such leaves [Bonner & Thurlow (24)]. The small amount of information which we do have on the relation of auxin level to photoperiodic regime is however in agreement with the view that leaf auxin levels drop during the night and rise during the day [see summary in (22)].

The pineapple.—The pineapple is remarkable in that the initiation of flowering is elicited by application of minute amounts of such active auxins as NAA or 2,4-D (36, 41, 128). Larger amounts of these same substances tend to suppress flowering (41). On the other hand, the pineapple normally contains an active auxin, IAA (56, 57, 131), and flowering, at least as induced by treatment with ethylene, is associated with a decrease in this normal auxin level [Leopold in (129)]. How can these two sets of facts be reconciled? An interesting property of the pineapple brought out by Cooper (41) is its apparent lack of flowering response to applied IAA. Although NAA induced flowering in Cooper's experiments, IAA was ineffective at all concentrations tested. In all of the work on pineapple thus far reported in the literature, synthetic auxins rather than IAA have been used for flower initiation. It appears as a bare possibility, then, that IAA may actually be devoid of flower-inducing properties in the pineapple and may possess only inhibitory effects on the process as it does in certain other species. This would suggest that in the pineapple we may have a species for which NAA and other synthetic materials act as antagonists to the native auxin of the plant. According to this view, NAA, or 2,4-D at appropriately low concentrations would act to block the action of the native IAA, much as TIBA appears to block action of the native auxin in such short-day plants as *Xanthium* or *Kalanchoe*. It would be of interest to know whether the flower-inducing action of NAA or 2,4-D on pineapple can be inhibited by the simultaneous application of IAA, just as the action of TIBA on *Xanthium* can be blocked by added IAA. The

hypothesis concerning auxin relations of flowering in the pineapple here proposed is a purely speculative one. Its element of attractiveness lies in the fact that, according to the hypothesis, the auxin relations of flowering in the pineapple would be consistent with those of other plants in so far as we at present understand these relations. Its element of improbability lies in the fact that we have thus far no basis for the view that NAA or 2,4-D may behave as active auxins in one species and as auxin antagonists (at very low concentrations) in other species.

STUDIES OF AUXIN ACTION

The ability of very small amounts of applied auxin to produce profound changes in treated plants as well as the great diversity of effects which are induced by auxin treatment suggests that auxin may exert its effect on some general and basic metabolic process. According to this view, the observed response, whether growth by elongation, production of roots, suppression of lateral buds or of flowering, increase in rate of protoplasmic streaming, or production of changes in chemical composition would simply be the visible manifestation of the effects of the hormone on this common basic process. This view would suggest that auxin may function as an essential ingredient of some particular enzymatic process central to the plant's economy. A great amount of effort has been devoted to attempts to discover the mechanism by which auxin exerts its action and, in particular, to discover the basic auxin-regulated process which has been envisaged. Among the varied approaches to the subject which have been used, the following are of particular interest:

- (a) Studies of the effects of growth substances on individual plant enzyme systems. In such experiments, the auxin may be applied either to the intact tissue prior to isolation of the enzyme or it may be added directly to the reaction mixture with the isolated enzyme.
- (b) Determination of the changes in chemical composition of the plant induced by applied auxin.
- (c) Studies of the relations between growth and respiration.
- (d) Studies of the process of water uptake and the effect of auxins on this process.

We propose to review briefly the general picture developed in each of these lines of inquiry.

EFFECTS OF AUXINS ON INDIVIDUAL ENZYME SYSTEMS

Table II summarizes the information on the effects of auxin on a number of isolated individual plant enzymes or enzyme systems. Most investigations in this field have been concerned with oxidative or other respiratory enzymes. It is clear even from the limited data of Table II that the activities of numerous enzymes are influenced by growth substances added *in vitro*, some being increased, some inhibited. It is difficult to demonstrate that an effect of auxin on a particular enzyme *in vitro* bears any intimate relation to

TABLE II
EFFECT OF AUXINS ON ISOLATED ENZYME SYSTEMS*

Enzyme	Source	Auxin	Effect	Reference
Aldolase	pea seed	IAA	0	Stumpf 1948 (110)
Alcohol dehydrogenase	Avena coleoptile	IAA and NAA	0	Berger & Avery 1943 (10)
Ascorbic acid oxidase	barley seedlings	10^{-3} M IAA, IPA, IBA NAA, 2,4,5-T, 2,4-D	8 to 40% inhibition	Miller & Burris 1951 (89)
Ascorbic acid oxidase	bean leaf	10^{-3} M 2,4,5-T, MCPA 2,4-D, NAA, IAA, IPA; IBA	5 to 30% inhibition	Wagenknecht <i>et al.</i> 1951 (136)
Catalase	castor bean	2,4-D	0	Hagen 1949 (63)
Fumarase	Avena coleoptile	IAA and NAA	0	Berger & Avery 1943 (10)
Glutamic dehydrogenase	Avena coleoptile	IAA 1000 mg./l. IBA 0 to 100 mg./l. NAA 1000 mg./l. NAAmide 0 to 100 mg./l.	50% inhibition 0 20% inhibition 0	Berger & Avery 1944 (9)
Glycolic acid oxidase	bean leaf	5×10^{-4} to 1×10^{-3} M 2,4-D, 2,4,5-T, IAA IPA; IBA, MCPA	0	Wagenknecht <i>et al.</i> 1951 (136)
Glycolic acid oxidase	barley	10^{-4} M IAA, IPA, NAA; 2,4-D, 2,4,5-T, MCPA	10 to 20% inhibition	Miller & Burris 1951 (89)
IAA oxidase	pea epicotyls	2,4-D 5×10^{-3} to 5×10^{-2} M	stimulation	Goldacre 1949 (54)
Isocitric dehydrogenase	Avena coleoptile	IAA 0.05 to 1000 mg./l. IBA and NAA 0.05 to 100 mg./l. NAAmide 100 mg./l. NAAmide 1000 mg./l.	0 0 30% inhibition 50% inhibition	Berger & Avery 1944 (9)
α -hydroxyacid oxidase	castor bean seed	2,4-D	0	Hagen 1949 (63)
Lipase	castor bean seed	1.5×10^{-3} M 2,4-D	50% inhibition	Hagen 1949 (63)
Lipase	wheat germ	5.6×10^{-3} M 2,4-D	50% inhibition	Kvamme 1949 (72)
Malic dehydrogenase	Avena coleoptile	NAAmide 1000 mg./l. IAA 1000 mg./l. NAA 1000 mg./l. IBA 1000 mg./l.	inhibition inhibition inhibition inhibition	Berger & Avery 1943 (10)
Phosphoglyceric acid kinase	pea seed meal	IAA 10^{-3} M	0	Axelrod & Bandurski (unpublished)
Phosphohexokinase	pea seed meal	IAA 5 mg./l.	0	Axelrod, Saltman, Bandurski & Baker (unpublished)
Polyphenol oxidase	castor bean seed	2,4-D	0	Hagen 1949 (63)
Glycolic acid oxidase	barley	10^{-4} M, IAA, IPA, NAA; 2,4-D, 2,4,5-T, MCPA	10 to 20% inhibition	Miller & Burris 1951 (89)

* Abbreviations:

IAA—Indoleacetic acid
IBA—Indole butyric acid
NAA— α -naphthaleneacetic acid
NAAmide— α -naphthaleneacetimide

2,4-D—2,4-dichlorophenoxyacetic acid
IPA—Indolepropionic acid
2,4,5-T—trichlorophenoxyacetic acid
MCPA—2-methyl-4-chlorophenoxyacetic acid

the effects of auxin in eliciting physiological responses. To establish such a relationship, one might (a) demonstrate similar requirements as to structural specificity, (b) correlate the sensitivity of the enzyme to auxin with the

sensitivity to auxin of the species from which the enzyme is extracted, and (c) show that effects on the enzymic reaction occur over the same range of auxin concentrations which influence plant growth and similar physiological responses. It has not been shown for any of the cases reported in Table II that these criteria are fulfilled and we do not know, therefore, that any of the effects in Table II are of physiological interest.

Treatment of plant tissue with auxin has resulted in several interesting effects upon the activities of the subsequently extracted enzymes. The first such effect to be reported was that of Berger & Avery (11) on the alcohol and malic dehydrogenases of *Avena* coleoptile tissue. Increases in activity of the order of 200 and 150 per cent, respectively, were obtained. These effects develop much more slowly than the growth response and are presumably secondary to it. No effects were found under the same conditions on glutamic, citric, or isocitric dehydrogenase. Gall (47) observed that bean stem sections cultured on starch-agar in the presence of 2,4-D degrade the starch contained in the media over a considerably greater area than untreated sections. Presumably the 2,4-D-treated, and, therefore, growing tissue possesses greater soluble amylase or phosphorylase activity than does the control tissue. Alternatively, the permeability of the cell membranes to these enzymes might be increased. Newcomb (93) has obtained large increases in the ascorbic acid oxidase activity (fresh weight basis) of a particulate fraction isolated from tobacco pith sections as a result of culturing the sections in the presence of 3.5 mg./l. IAA. The increase precedes the growth and respiratory changes induced by the growth substance treatment. The author therefore suggests that ascorbic acid oxidase activity may be causally related to growth. It is of interest that neither cytochrome oxidase nor tyrosinase showed any marked response to the auxin treatment.

Galston (52) has reported a marked diminution of the catalase content of *Helianthus tuberosus* tissue as a result of culture in the presence of 2,4-D or IAA. Further, normal tissues of *Scorzonera*, *Vitis*, and *Parthenocissus* contained more catalase than did habituated tissues which, in turn, contained more catalase than did crown gall tissue. There was thus an inverse correlation between the growth of the tissue and the content of catalase. Olsen (94) has shown that the phosphatase activity of *Zea* roots increases strikingly as a result of culture in 1.5 p.p.m. 2,4-D solution, a concentration which also causes an inhibition of root growth. The effects were apparent in as short a time as 3 hr.

It is clear, then, that treatment of plant tissues with auxin results in effects on a wide variety of enzymes; the activities of some are increased while the activities of others are decreased. It is, however, difficult to assess the physiological significance of such effects since in most cases a comparison is being made between growing and nongrowing tissue. It is not clear that there is any direct relation between these effects and the mechanism of auxin action. Aside from this, however, the results are valuable in so far as they serve to more rigorously define growth and to give us a more detailed description of the chemical changes which occur during this process.

AUXIN-INDUCED CHANGES IN PLANT COMPOSITION

Changes in chemical composition of the plant, induced by application of herbicidal concentrations of growth-regulating substances, have recently been reviewed by Mitchell (90) and by Blackman *et al.* (12) and will not be reviewed here. Investigations of changes in chemical composition which attend the induction of growth by auxin, a subject much studied in the past, have recently been intensified by Christiansen & Thimann and by Burström. The experiments of Christiansen & Thimann were intended as an all-out effort to follow every chemical change which occurs in plant tissue during the response to growth substance. A portion of this work has been reviewed by Thimann, Bonner & Christiansen (122) and by Thimann (116). Their experimental material consisted of 20 mm. pea stem sections incubated for 24 hr. in water or IAA at 1 mg./l. The control sections incubated in water increase in length by 20 per cent and in fresh weight by 22 per cent as contrasted to a 50 per cent increase in length and 60 per cent increase in fresh weight for the auxin-treated sections [Christiansen & Thimann (32)]. Both treated and control sections lose approximately 11 per cent of their initial dry weight. The auxin-treated sections respire 15 to 30 per cent faster (dry weight basis) than the control sections. About 25 per cent of the reducing sugars initially present are consumed both by the control and auxin-treated sections. Over 70 per cent of the sucrose initially present is consumed by the auxin-treated sections, while only 57 per cent is lost by the controls. Starch and dextrans are not present in analytically detectable amounts in the pea tissue. No changes were found in the organic phosphorous fraction as a whole. The methods used did not, however, differentiate between the individual constituents of the organic phosphorous fraction. A slight increase in cell wall constituents occurs during growth. Polysaccharides and polyuronides compose up to 23 per cent of the initial dry weight. This increases to 27 per cent for the auxin-treated sections and to 25 per cent for the control sections. There appears to be a slightly greater consumption of organic acids and of fats in the treated sections [Christiansen & Thimann (33)]. Slightly more of the amino acids initially present are consumed [Christiansen & Thimann (34)] and slightly more protein synthesized by treated than by untreated sections. Christiansen & Thimann conclude that these transformations of nitrogenous substances are of critical significance for growth.

Studies by Boroughs (27) and by Burström (31), in contrast to those of Christiansen & Thimann, have failed to find any direct relation of protein synthesis to growth. Boroughs, in an elegant study, followed the influence of auxin on the rate of protein turnover in corn coleoptile sections as determined by the rate of incorporation of C^{14} -labeled glycine and leucine into the plant proteins. The coleoptiles were incubated in media containing 3 per cent sucrose (0.03 *M*), K maleate (pH 4.6), the carboxyl-labeled amino acid (0.002 *M*), and with or without 3 mg./l. IAA. Growth, total protein, and specific activity of protein were measured at intervals up to 24 hr. No appreciable net synthesis of protein occurred either in the presence or absence

of IAA. The rate at which the radioactivity initially supplied in the form of amino acids appeared in the protein of the sections was similarly independent of the presence or absence of growth substance. Auxin did not therefore alter the rate of protein synthesis, nor did it influence the turnover rate of the protein. In the work of Burström (31), the growth of wheat roots was followed in the presence or absence of *p*-chlorophenoxyisobutyric acid (PCIB). Treatment with this auxin antagonist resulted, for example, in an 88 per cent increase in length (in 10^{-6} M PCIB) with only an 8 per cent increase in protein nitrogen. From the lack of proportionality between growth and protein synthesis, Burström concludes that the processes are not intimately linked.

In summary, the chemical changes associated with auxin-induced growth are small. This applies, for example, to the changes in organic acids, amino acids, carbohydrates, and total dry weight. It had previously been shown that an increase in cell wall constituents is not an obligatory accompaniment of growth [Bonner (14)]. The absence of protein synthesis during section growth observed by Boroughs (27) and the lack of proportionality of protein synthesis and growth observed by Christiansen & Thimann (34) and by Burström (31) would further indicate that protein synthesis is not a necessary accompaniment of growth. Thus, from a quantitative standpoint, there is but one striking chemical effect of auxin treatment which is common to all the experiments described above. This is the effect of auxin upon the uptake of water by the tissue. So far as the experiments reviewed are concerned, the only meaningful metabolic difference between a growing and a nongrowing section would appear to be the uptake of water by the growing section.

RESPIRATION AND GROWTH

Much of the work on the mechanism of auxin action has involved attempts to elucidate the nature of the connection between respiration and auxin-induced growth responses. Several recent reviews have considered this relationship [Audus (4); Avery (5); Bonner & Wildman (26); and Thimann (116)]. That growth in response to auxin is in fact obligatorily coupled to oxidative metabolism has been known for many years [Bonner (13)]. There remain, however, a number of questions concerning the nature of the energetic coupling—questions which are common to animal and plant physiology. Two experimental approaches to the study of the linkage between respiration and growth have been utilized. The characteristics of the auxin-induced increase in respiration may be studied, or the effects of varied respiratory inhibitors on growth may be investigated in an attempt to determine the point of auxin action.

That the same range of concentrations of auxin which effect growth may also increase respiration is well established [Berger & Avery (8); Bonner (17); Christiansen & Thimann (33); Commoner & Thimann (40); Michel (86)]. It is also true, however, that increased respiration does not necessarily accompany the auxin-induced growth response [Bonner (15)]. There is there-

fore no constant and uniform stoichiometry between auxin supply and respiration.

In theory it should be possible to discover how auxin increases the respiratory rate by finding out first what is the rate-limiting factor in the absence of auxin. This limiting factor does not appear to be either the substrate concentration or the capacity of the respiratory enzyme system (25). Of particular interest in this connection are the studies of the effect of auxin on the isolated mitochondrial respiratory system. By appropriate procedures, it is possible to separate from plant tissues an enzyme complex, associated with the mitochondria, which is capable of oxidizing pyruvate to CO_2 and water [Millerd *et al.* (88)]. These isolated particles are capable of accounting for the bulk of the respiration of the stems of seedlings such as mung bean [Millerd (87)]. The rate at which pyruvate is oxidized *in vitro* by the isolated mitochondrial system is not influenced by added auxin [Price, Bonner & Millerd (96)] nor is the capacity of the mitochondria for pyruvate oxidation influenced by pretreatment of the living plant with IAA. The failure to obtain increased respiration with the isolated system is by no means conclusive evidence that auxin does not act in this portion of the metabolism. It is, however, in agreement with other evidence which suggests that the respiration-limiting and auxin-influenced step in seedling tissues, such as those of the *Avena* coleoptile, is concerned with the phosphate transfer mechanism.

A second approach to the study of the relation between auxin-induced growth and respiration is based upon the experimental disassociation of the two processes by the use of inhibitors. Thus, certain substances inhibit growth and respiration to the same extent. This is true of cyanide (13, 15). It would appear then that the electron transfer portion of respiration is essential to growth and that inhibition of this electron transfer results in proportional growth inhibition. Other inhibitors selectively influence growth, leaving respiration unaffected or affected only to a lesser extent. Thus, for example, arsenate at a concentration of 30 mg./l. inhibits growth by 94 per cent while respiration continues unaffected (20) Canavanine at a concentration of 50 mg./l. is not inhibitory to endogenous respiration but inhibits the increase in respiration elicited by IAA (16) and inhibits growth by 90 per cent (17). Fluoride is also a selective inhibitor of growth [Bonner & Wildman (26); Christiansen & Thimann (33)]. Similarly, Christiansen & Thimann (33) have shown that concentrations of iodoacetate and of arsenite sufficient to inhibit growth 50 per cent, inhibit respiration to the extent of 31 and 19 per cent, respectively. There are no known inhibitors which inhibit respiration without inhibiting growth. We must conclude that there are steps in the auxin-induced growth process and, indeed, in the mechanism by which auxin increases the respiratory rate, which are more sensitive to certain inhibitors than is respiratory gas exchange. This again suggests the conclusion arrived at earlier in this section that the effect of auxin is not directly concerned with either the electron transfers or carbon transformations of respiration.

A deeper insight into the relationship between growth and respiration has been obtained by the use of the inhibitor 2,4-dinitrophenol (DNP). This substance may inhibit growth almost completely while causing an actual increase in the rate of respiration. Thus, for example, a concentration of 5 mg./l. DNP will inhibit growth by 88 per cent while actually increasing respiration by 38 per cent [Bonner (16)]. A similar stimulation of respiration with DNP has been observed by Kelly (70). We have sufficient knowledge of the action of DNP on plants to make possible an interpretation of the effects of DNP on growth and respiration. DNP is known to inhibit the synthesis of adenosine triphosphate (ATP) which is normally coupled to the respiratory oxidations. In tissues in which the capacity of the phosphorylating system limits respiratory rate, this rate is actually increased by uncoupling with DNP [Loomis & Lipmann (85)]. Reactions which are driven by ATP and which are dependent upon respiration for this ATP are, however, inhibited. Among the many well-established examples of energy-requiring processes which are inhibited by DNP are enzyme synthesis [Sussman & Spiegelman (111)], HCl secretion by oxyntic cells [Davies (42)], and cell division [Clowes & Krah1 (37)]. Auxin-induced growth is not the only plant process which is known to be inhibited by DNP. Robertson (102) found that concentrations of DNP which inhibit active salt uptake by roots stimulate oxygen uptake. A similar observation was made by Stenlid (108) for the absorption of glucose by wheat roots, while Hackett & Thimann (62) found that DNP inhibits water uptake by potato discs. Recent work on oxidative phosphorylation and the synthesis of ATP by plants has shown that energetic coupling in the plant is much as it is in animals. The respiratory oxidations of isolated plant mitochondria are linked to the uptake of *ortho* phosphate and the synthesis of ATP [Millerd (87); Millerd *et al.* (88)]. The synthesis of ATP by plant mitochondria is inhibited by DNP [Bonner & Millerd (23)]. It would, therefore, appear that the inhibition of growth by DNP may be explained by the necessity of ATP for growth. Since auxin exerts no effect on respiration in the presence of DNP, arsenate, or other uncoupling agents, it follows that the influence of auxin on respiration is exerted through that part of the system which is uncoupled and hence inactive in the presence of such agents.

How then may we picture the mechanism by which auxin increases the respiratory rate? It would appear that the rate of respiration of the *Avena* coleoptile, for example, is normally limited by the capacity of the phosphorylating system. This is indicated not only by the fact that uncoupling with DNP increases the rate of respiration but also by the fact that the addition of adenylic acid to the plant increases the respiratory rate (17). Since auxin increases respiration in a system whose rate limiting step is the phosphorylating one, it appears logical to conclude that auxin must in some manner affect this phosphorylative process.

The evidence which has been presented indicates that, at least under the conditions of short-term section experiments, the relationship between respiration and growth may consist in a dependency of the growth process upon

the ATP produced in respiration. What then is the nature of this ATP-requiring growth process?

WATER UPTAKE

Considerations presented in an earlier section have indicated that, of all the chemical changes during auxin-induced growth, the most spectacular is that concerned with water uptake. We have further seen that at least in the case of growth by elongation, the dependency of growth upon respiration seems to lie in a requirement of growth for ATP. It will be of interest to consider the possibility that water uptake may be the respiration- and ATP-requiring process of auxin-induced growth. Since there is no large and consistent effect of auxin in increasing respiration, it would appear necessary to assume that the effect of auxin would be either directly on the process of water uptake or upon the linkage of ATP to this process.

That auxin does influence water uptake by plant tissue has been recognized since the early work of Reinders (99, 100), of Commoner *et al.* (38, 39), and of Van Overbeek (127), all of which were concerned with the role of auxin in water uptake by potato tuber discs. That the effect is a result of an active secretion of water into the cell interior was indicated by Commoner & co-workers (38) who found that auxin can induce water uptake in discs immersed in plasmolyzing concentrations of sucrose. Commoner & Mazia (39) further found that auxin stimulates the uptake of KCl and in the same range of concentrations which increase water uptake. They concluded that auxin brings about an active water uptake and does so by increasing the absorption of osmotically active substances such as salts. Van Overbeek (127) has, however, shown that the osmotic concentration of the cell contents as measured cryoscopically actually decreases during response to auxin. This fact, together with the observation of Reinders (100) that auxin effects can be obtained even with tissues immersed in essentially pure water, caused Van Overbeek to conclude that the effect of auxin on water uptake must be exerted through some mechanism other than an osmotic one—presumably by active water secretion.

That such active water uptake, whose existence was early suggested by experiments of Bennet-Clark *et al.* (6), may depend intimately on respiration has been shown by Steward *et al.* (109), who found a reasonably direct relationship between the water uptake and the respiration rate of potato discs. Reinders (99, 100) made similar observations with potato tuber discs and discs of other storage tissues. Van Overbeek (126) showed that 50 to 70 per cent of the root pressure of decapitated tomato plants apparently results from an active uptake of water, which is respiration-dependent and cyanide-inhibitable.

There is thus a considerable body of evidence indicating the existence of a metabolically controlled and dependent water uptake. In order to take up water against a gradient, metabolic energy must be expended by the cell. Levitt (78) has calculated that an appreciable gradient in water concentra-

tion across the cell boundary may be maintained by the energy liberated in respiration. That water uptake is inhibited by DNP has been shown not only for the case of auxin-controlled growth in length but also for the case of auxin-induced water uptake by potato discs [Hackett & Thimann (62)]. This suggests that active water uptake, in so far as it takes place, may be powered in some way by ATP. The process of water uptake by sections and by discs of storage tissue is increased by auxin and it appears that salt uptake may be similarly influenced [Commoner (39)]. We have seen that the analytically detectable effect of auxin on metabolites other than water are slight indeed. Our attention has thus been directed to the possibility that auxin may serve to couple respiration to the process of water accumulation. The study of whether this is indeed the primary effect of auxin will undoubtedly provide a profitable field for future investigation.

LITERATURE CITED

1. Aberg, B., *Physiol. Plantarum*, **3**, 447 (1950)
2. Ashby, W. C., *Botan. Gaz.*, **112**, 237 (1951)
3. Atkinson, D., *Frasch Foundation Ann. Rept.* (Calif. Inst. Technol., Pasadena, Calif., 50 pp., 1950)
4. Audus, L. J., *Biol. Revs., Cambridge Phil. Soc.*, **24**, 51 (1949)
5. Avery, G. S., Jr., *Plant Growth Substances*, 105 (Univ. of Wis. Press, Madison, Wis., 476 pp., 1951)
6. Bennett-Clark, J. A., Greenwood, A. D., and Barker, J. W., *New Phytologist*, **35**, 277 (1936)
7. Bentley, J. A., *Nature*, **165**, 449 (1950)
8. Berger, J., Smith P., and Avery, G. S., *Am. J. Botany*, **33**, 601 (1946)
9. Berger, J., and Avery, G. S., *Am. J. Botany*, **31**, 11 (1944)
10. Berger, J., and Avery, G. S., *Am. J. Botany*, **30**, 297 (1943)
11. Berger, J., and Avery, G. S., *Science*, **98**, 454 (1943)
12. Blackman, G. E., Templeman, W. G., and Halliday, D. J., *Ann. Rev. Plant Physiol.*, **2**, 199 (1950)
13. Bonner, J., *J. Gen. Physiol.*, **17**, 63 (1933)
14. Bonner, J., *Proc. Natl. Acad. Sci.*, **20**, 393 (1934)
15. Bonner, J., *J. Gen. Physiol.*, **20**, 1 (1936)
16. Bonner, J., *Am. J. Botany*, **36**, 323 (1949)
17. Bonner, J., *Am. J. Botany*, **36**, 429 (1949)
18. Bonner, J., *Botan. Gaz.*, **110**, 625 (1949)
19. Bonner, J. (Unpublished data)
20. Bonner, J., *Plant Physiol.*, **25**, 181 (1950)
21. Bonner, J., and Galston, A. W., *Botan. Gaz.*, **106**, 185 (1944)
22. Bonner, J., and Liverman, J., *Growth and Differentiation in Plants* (Am. Soc. Plant Physiol. Monograph in press)
23. Bonner, J., and Millerd, A. (Unpublished data)
24. Bonner, J., and Thurlow, J., *Botan. Gaz.*, **110**, 613 (1949)
25. Bonner, J., and Wildman, S. G., *Arch. Biochem.*, **10**, 497 (1946)
26. Bonner, J., and Wildman, S. G., *Growth*, **10** (Suppl.), 51 (1946)
27. Boroughs, H., Paper presented to Am. Soc. Plant Physiol. (Western Section), (Los Angeles, 1951)

28. Bourne, M. C., and Young, L., *Biochem. J.*, **28**, 805 (1934)
29. Burström, H., *Physiol. Plantarum*, **3**, 277 (1950)
30. Burström, H., *Physiol. Plantarum*, **4**, 470 (1951)
31. Burström, H., *Physiol. Plantarum*, **4**, 199 (1951)
32. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **26**, 230 (1950)
33. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **26**, 248 (1950)
34. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **28**, 117 (1950)
35. Cholodny, N. G., *Herbage Revs.*, **7**, 223 (1939)
36. Clark, H., and Kearns, K., *Science*, **95**, 536 (1942)
37. Clowes, G. H. A., and Krahl, M. E., *J. Gen. Physiol.*, **20**, 145 (1936)
38. Commoner, B., Fogel, S., and Muller, W. H., *Am. J. Botany*, **30**, 23 (1943)
39. Commoner, B., and Mazia, D., *Plant Physiol.*, **17**, 682 (1942)
40. Commoner, B., and Thimann, K. V., *J. Gen. Physiol.*, **24**, 279 (1941)
41. Cooper, W. C., *Proc. Am. Soc. Hort. Sci.*, **41**, 93 (1942)
42. Davis, R. E., *Biol. Revs. Cambridge Phil. Soc.*, **26**, 87 (1951)
43. de Waard, J., and Roodenburg, J. W. M., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **51**, 248 (1948)
44. de Waard, J., and Florschütz, P. A., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **51**, 1317 (1948)
45. Dostal, R., and Hosek, M., *Flora*, **131**, 263 (1937)
46. Esteves-de-Sousa, A., *Portuguesa Acta Biolog.*, **3**, 91 (1950)
47. Gall, H. J. F., *Botan. Gaz.*, **110**, 319 (1948)
48. Galston, A. W., *The Physiology of Flowering with Special Reference to Floral Initiation in Soybeans* (Doctoral thesis, Univ. of Illinois, Urbana, Ill., 1943)
49. Galston, A. W., *Am. J. Botany*, **34**, 356 (1947)
50. Galston, A. W., *Am. J. Botany*, **34**, 356 (1947)
51. Galston, A. W., *Science*, **111**, 619 (1950)
52. Galston, A. W., *Compt. rend.*, **232**, 1505 (1951)
53. Galston, A. W., and Baker, R. S., *Am. J. Botany*, **38**, 190 (1951)
54. Goldacre, P. L., *Australian J. Sci. Research*, [B] **2**, 154 (1949)
55. Goldstein, A., *J. Gen. Physiol.*, **27**, 529 (1944)
56. Gordon, S. A., and Sanchez-Nieva, F., *Arch. Biochem.*, **20**, 356 (1949)
57. Gordon, S. A., and Sanchez-Nieva, F., *Arch. Biochem.*, **20**, 367 (1949)
58. Gordon, S. A., and Weber, R. P., *Plant Physiol.*, **26**, 192 (1951)
59. Gorter, C., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **52**, 1185 (1949)
60. Green, M., and Fuller, H., *Science*, **108**, 415 (1948)
61. Haagen-Smit, A. J., Dandliker, W. B., Wittwer, S. H., and Murneek, A. E., *Am. J. Botany*, **33**, 118 (1946)
62. Hackett, D., and Thimann, K. V., *Plant Physiol.*, **25**, 648 (1950)
63. Hagen, C. E., Claggett, C. O., and Helgeson, E. A., *Science*, **110**, 116 (1949)
64. Holley, R. W., Boyle, F. P., Durfee, H. K., and Halley, A. D., *Arch. Biochem.*, **32**, 192 (1951)
65. Hamner, K. C., and Bonner, J., *Botan. Gaz.*, **100**, 388 (1938)
66. Hansch, C., and Muir, R. M., *Plant Physiol.*, **25**, 389 (1950)
67. Hansch, C., Muir, R. M., and Metznerberg, R. L., *Plant Physiol.*, **26**, 812 (1951)
68. Harder, R., and van Senden, H., *Naturwissenschaften*, **36**, 348 (1949)
69. Hitchcock, A. E., and Zimmerman, P. W., *Contrib. Boyce Thompson Inst.*, **16**, 225 (1951)
70. Kelly, S., and Avery, G. S., Jr., *Am. J. Botany*, **36**, 421 (1949)

71. Koepfli, J. B., Thimann, K. V., and Went, F. W., *J. Biol. Chem.*, **122**, 763 (1937)
72. Kvamme, J., Clagett, C. O., and Treumann, B., *Arch. Biochem.*, **24**, 321 (1949)
73. Laibach, F., and Kribben, F., *Naturwissenschaften*, **37**, 114 (1950)
74. Larsen, P., *Dansk Botan. Arkiv*, **11**, 1 (1944)
75. Larsen, P., *Am. J. Botany*, **36**, 32 (1949)
76. Larsen, P., *Plant Physiol.*, **26**, 697 (1951)
77. Leopold, C., and Thimann, K. V., *Am. J. Botany*, **36**, 342 (1949)
78. Levitt, J., *Plant Physiol.*, **23**, 505 (1948)
79. Li, C. H., *Inhibition and Induction of Flowering in Higher Plants by Application of Auxins and Other Substances* (Master's thesis, Calif. Inst. Technol., Pasadena, Calif., 1949)
80. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, **56**, 658 (1934)
81. Link, G. K. K., Egger, S. V., and Moulton, J., *Botan. Gaz.*, **101**, 928 (1940)
82. Linser, H., *Planta*, **39**, 377 (1951)
83. Liverman, J., Paper presented to Am. Soc. Plant Physiol. (Los Angeles, 1951)
84. Lona, F., *Rend. inst. lombardo di scienze*, **83**, 1 (1950)
85. Loomis, W. F., and Lipmann, F., *J. Biol. Chem.*, **173**, 807 (1948)
86. Michel, B. E., *Botan. Gaz.*, **112**, 418 (1951)
87. Millerd, A., *Respiratory Oxidation and Energy Transfer by Plant Systems* (Doctoral thesis, Calif. Inst. Technol., Pasadena, Calif., and Univ. of Sydney, Sydney, Australia, 1951)
88. Millerd, A., Bonner, J., Axelrod, B., and Bandurski, R., *Proc. Natl. Acad. Sci.*, **37**, 855 (1951)
89. Miller, I. H., and Burris, R. H., *Am. J. Botany*, **38**, 547 (1951)
90. Mitchell, J. W., *Plant Growth Substances*, 141 (Univ. of Wis. Press, Madison, Wis., 476 pp., 1951)
91. Muir, R. M., and Hansch, C., *Plant Physiol.*, **26**, 369 (1951)
92. Muir, R. M., Hansch, C., and Gallup, A. H., *Plant Physiol.*, **24**, 359 (1949)
93. Newcomb, Eldon H., *Proc. Soc. Exptl. Biol. Med.*, **76**, 504 (1951)
94. Olsen, K., Paper presented to Am. Soc. Plant Physiol. (Western Section), (Salt Lake City, 1950)
95. Osborne, D. J., and Wain, R. L., *J. Hort. Sci.*, **26**, 60 (1950)
96. Price, C., Bonner, J., and Millerd, A. (Unpublished data)
97. Quastel, J. H., *Biochem. J.*, **20**, 166 (1926)
98. Redemann, C. T., Wittwer, S. H., and Sell, H. M., *Arch. Biochem. Biophys.*, **32**, 80 (1951)
99. Reinders, D. E., *Proc. Kon. Akad. V. Wetenschap. Amsterdam*, **41**, 820 (1938)
100. Reinders, D. E., *Rec. trav. botan. néerland.*, **39**, 1 (1942)
101. Reinert, J., *Z. Naturforsch.*, **56**, 374 (1950)
102. Robertson, R. N., *Ann. Rev. Plant Physiol.*, **2**, 1 (1951)
103. Skoog, R., Schneider, C., and Malan, P., *Am. J. Botany*, **29**, 568 (1942)
104. Snyder, W. E., *Plant Physiol.*, **24**, 195 (1949)
105. Strauss, O. H., and Goldstein, A., *J. Gen. Physiol.*, **26**, 259 (1943)
106. Stehsel, M. (Unpublished data)
107. Stehsel, M., and Wildman, S. G., *Abstracts Ann. Meeting, Botan. Soc. Am. (Physiol. Section)*, (Columbus, Ohio, 1950)
108. Stenlid, G., *Physiol. Plantarum*, **2**, 350 (1949)
109. Steward, F. C., Stout, P. R., and Preston, C., *Plant Physiol.*, **15**, 409 (1940)
110. Stumpf, P. K., *J. Biol. Chem.*, **176**, 233 (1948)

28. Bourne, M. C., and Young, L., *Biochem. J.*, **28**, 805 (1934)
29. Burström, H., *Physiol. Plantarum*, **3**, 277 (1950)
30. Burström, H., *Physiol. Plantarum*, **4**, 470 (1951)
31. Burström, H., *Physiol. Plantarum*, **4**, 199 (1951)
32. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **26**, 230 (1950)
33. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **26**, 248 (1950)
34. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **28**, 117 (1950)
35. Cholodny, N. G., *Herbage Revs.*, **7**, 223 (1939)
36. Clark, H., and Kearns, K., *Science*, **95**, 536 (1942)
37. Clowes, G. H. A., and Krahle, M. E., *J. Gen. Physiol.*, **20**, 145 (1936)
38. Commoner, B., Fogel, S., and Muller, W. H., *Am. J. Botany*, **30**, 23 (1943)
39. Commoner, B., and Mazia, D., *Plant Physiol.*, **17**, 682 (1942)
40. Commoner, B., and Thimann, K. V., *J. Gen. Physiol.*, **24**, 279 (1941)
41. Cooper, W. C., *Proc. Am. Soc. Hort. Sci.*, **41**, 93 (1942)
42. Davis, R. E., *Biol. Revs. Cambridge Phil. Soc.*, **26**, 87 (1951)
43. de Waard, J., and Roodenburg, J. W. M., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **51**, 248 (1948)
44. de Waard, J., and Florschütz, P. A., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **51**, 1317 (1948)
45. Dostal, R., and Hosek, M., *Flora*, **131**, 263 (1937)
46. Esteves-de-Sousa, A., *Portuguesa Acta Biolog.*, **3**, 91 (1950)
47. Gall, H. J. F., *Botan. Gaz.*, **110**, 319 (1948)
48. Galston, A. W., *The Physiology of Flowering with Special Reference to Floral Initiation in Soybeans* (Doctoral thesis, Univ. of Illinois, Urbana, Ill., 1943)
49. Galston, A. W., *Am. J. Botany*, **34**, 356 (1947)
50. Galston, A. W., *Am. J. Botany*, **34**, 356 (1947)
51. Galston, A. W., *Science*, **111**, 619 (1950)
52. Galston, A. W., *Compt. rend.*, **232**, 1505 (1951)
53. Galston, A. W., and Baker, R. S., *Am. J. Botany*, **38**, 190 (1951)
54. Goldacre, P. L., *Australian J. Sci. Research*, [B] **2**, 154 (1949)
55. Goldstein, A., *J. Gen. Physiol.*, **27**, 529 (1944)
56. Gordon, S. A., and Sanchez-Nieva, F., *Arch. Biochem.*, **20**, 356 (1949)
57. Gordon, S. A., and Sanchez-Nieva, F., *Arch. Biochem.*, **20**, 367 (1949)
58. Gordon, S. A., and Weber, R. P., *Plant Physiol.*, **26**, 192 (1951)
59. Gorter, C., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **52**, 1185 (1949)
60. Green, M., and Fuller, H., *Science*, **108**, 415 (1948)
61. Haagen-Smit, A. J., Dandliker, W. B., Wittwer, S. H., and Murneek, A. E., *Am. J. Botany*, **33**, 118 (1946)
62. Hackett, D., and Thimann, K. V., *Plant Physiol.*, **25**, 648 (1950)
63. Hagen, C. E., Claggett, C. O., and Helgeson, E. A., *Science*, **110**, 116 (1949)
64. Holley, R. W., Boyle, F. P., Durfee, H. K., and Halley, A. D., *Arch. Biochem.*, **32**, 192 (1951)
65. Hamner, K. C., and Bonner, J., *Botan. Gaz.*, **100**, 388 (1938)
66. Hansch, C., and Muir, R. M., *Plant Physiol.*, **25**, 389 (1950)
67. Hansch, C., Muir, R. M., and Metzberg, R. L., *Plant Physiol.*, **26**, 812 (1951)
68. Harder, R., and van Senden, H., *Naturwissenschaften*, **36**, 348 (1949)
69. Hitchcock, A. E., and Zimmerman, P. W., *Contrib. Boyce Thompson Inst.*, **16**, 225 (1951)
70. Kelly, S., and Avery, G. S., Jr., *Am. J. Botany*, **36**, 421 (1949)

71. Koepfli, J. B., Thimann, K. V., and Went, F. W., *J. Biol. Chem.*, **122**, 763 (1937)
72. Kvamme, J., Clagett, C. O., and Treumann, B., *Arch. Biochem.*, **24**, 321 (1949)
73. Laibach, F., and Kribben, F., *Naturwissenschaften*, **37**, 114 (1950)
74. Larsen, P., *Dansk Botan. Arkiv*, **11**, 1 (1944)
75. Larsen, P., *Am. J. Botany*, **36**, 32 (1949)
76. Larsen, P., *Plant Physiol.*, **26**, 697 (1951)
77. Leopold, C., and Thimann, K. V., *Am. J. Botany*, **36**, 342 (1949)
78. Levitt, J., *Plant Physiol.*, **23**, 505 (1948)
79. Li, C. H., *Inhibition and Induction of Flowering in Higher Plants by Application of Auxins and Other Substances* (Master's thesis, Calif. Inst. Technol., Pasadena, Calif., 1949)
80. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, **56**, 658 (1934)
81. Link, G. K. K., Egger, S. V., and Moulton, J., *Botan. Gaz.*, **101**, 928 (1940)
82. Linser, H., *Planta*, **39**, 377 (1951)
83. Liverman, J., Paper presented to Am. Soc. Plant Physiol. (Los Angeles, 1951)
84. Lona, F., *Rend. inst. lombardo di scienze*, **83**, 1 (1950)
85. Loomis, W. F., and Lipmann, F., *J. Biol. Chem.*, **173**, 807 (1948)
86. Michel, B. E., *Botan. Gaz.*, **112**, 418 (1951)
87. Millerd, A., *Respiratory Oxidation and Energy Transfer by Plant Systems* (Doctoral thesis, Calif. Inst. Technol., Pasadena, Calif., and Univ. of Sydney, Sydney, Australia, 1951)
88. Millerd, A., Bonner, J., Axelrod, B., and Bandurski, R., *Proc. Natl. Acad. Sci.*, **37**, 855 (1951)
89. Miller, I. H., and Burris, R. H., *Am. J. Botany*, **38**, 547 (1951)
90. Mitchell, J. W., *Plant Growth Substances*, 141 (Univ. of Wis. Press, Madison, Wis., 476 pp., 1951)
91. Muir, R. M., and Hansch, C., *Plant Physiol.*, **26**, 369 (1951)
92. Muir, R. M., Hansch, C., and Gallup, A. H., *Plant Physiol.*, **24**, 359 (1949)
93. Newcomb, Eldon H., *Proc. Soc. Exptl. Biol. Med.*, **76**, 504 (1951)
94. Olsen, K., Paper presented to Am. Soc. Plant Physiol. (Western Section), (Salt Lake City, 1950)
95. Osborne, D. J., and Wain, R. L., *J. Hort. Sci.*, **26**, 60 (1950)
96. Price, C., Bonner, J., and Millerd, A. (Unpublished data)
97. Quastel, J. H., *Biochem. J.*, **20**, 166 (1926)
98. Redemann, C. T., Wittwer, S. H., and Sell, H. M., *Arch. Biochem. Biophys.*, **32**, 80 (1951)
99. Reinders, D. E., *Proc. Kon. Akad. V. Wetenschap. Amsterdam*, **41**, 820 (1938)
100. Reinders, D. E., *Rec. trav. botan. néerland.*, **39**, 1 (1942)
101. Reinert, J., *Z. Naturforsch.*, **56**, 374 (1950)
102. Robertson, R. N., *Ann. Rev. Plant Physiol.*, **2**, 1 (1951)
103. Skoog, R., Schneider, C., and Malan, P., *Am. J. Botany*, **29**, 568 (1942)
104. Snyder, W. E., *Plant Physiol.*, **24**, 195 (1949)
105. Strauss, O. H., and Goldstein, A., *J. Gen. Physiol.*, **26**, 259 (1943)
106. Stehsel, M. (Unpublished data)
107. Stehsel, M., and Wildman, S. G., *Abstracts Ann. Meeting, Botan. Soc. Am. (Physiol. Section)*, (Columbus, Ohio, 1950)
108. Stenlid, G., *Physiol. Plantarum*, **2**, 350 (1949)
109. Steward, F. C., Stout, P. R., and Preston, C., *Plant Physiol.*, **15**, 409 (1940)
110. Stumpf, P. K., *J. Biol. Chem.*, **176**, 233 (1948)

111. Sussman, M., and Spiegelman, S., *Arch. Biochem.*, **29**, 85 (1950)
112. Synerholm, M. E., and Zimmerman, P. W., *Contrib. Boyce Thompson Inst.* **14**, 91 (1945)
113. Tang, Y. W., and Bonner, J., *Arch. Biochem.*, **13**, 11 (1947)
114. Tang, Y. W., and Bonner, J., *Am. J. Botany*, **35**, 570 (1948)
115. Teas, H. J., and Newton, A., *Plant Physiol.*, **26**, 494 (1951)
116. Thimann, K. V., *Growth*, **15** (In press)
117. Thimann, K. V., *Plant Growth Substances*, 21 (Univ. of Wis. Press, Madison, Wis., 476 pp., 1951)
118. Thimann, K. V., and Bonner, W. D., Jr., *Am. J. Botany*, **35**, 271 (1948)
119. Thimann, K. V., and Bonner, W. D., Jr., *Plant Physiol.*, **23**, 158 (1948)
120. Thimann, K. V., and Bonner, W. D., Jr., *Am. J. Botany*, **36**, 214 (1949)
121. Thimann, K. V., and Bonner, W. D., Jr., *Proc. Natl. Acad. Sci.*, **35**, 272 (1949)
122. Thimann, K. V., Bonner, W. D., Jr., and Christiansen, G. S., *Plant Growth Substances*, 97 (Univ. of Wis. Press, Madison, Wis., 476 pp., 1951)
123. Thompson, H. E., Swanson, C. P., and Norman, A. G., *Botan. Gaz.*, **107**, 476 (1946)
124. Thurlow, J., and Bonner, J., *Am. J. Botany*, **34**, 603 (1947)
125. Tumanov, I. I., and Lizandr, A. A., *J. botan. U.R.S.S.*, **31**, 20 (1946)
126. van Overbeek, J., *Am. J. Botany*, **29**, 677 (1942)
127. van Overbeek, J., *Am. J. Botany*, **31**, 265 (1944)
128. van Overbeek, J., *Science*, **102**, 621 (1945)
129. van Overbeek, J., *Plant Growth Substances*, 225 (Univ. of Wis. Press, Madison, Wis., 476 pp., 1951)
130. van Overbeek, J., Blondeau, R., and Horne, V., *Am. J. Botany*, **38**, 589 (1951)
131. van Overbeek, J., Gregory, L. E., and Velez, I., *Proc. Am. Soc. Hort. Sci.*, **47**, 434 (1946)
132. Veldstra, H., and Havinga, E., *Rec. trav. chim.*, **62**, 841 (1943)
133. Veldstra, H., and Havinga, E., *Enzymologia*, **11**, 373 (1945)
134. von Denffer, D., and Gründler, H., *Biol. Zentr.*, **69**, 272 (1950)
135. Wagenknecht, A. C., and Burris, R. H., *Arch. Biochem.*, **25**, 30 (1950)
136. Wagenknecht, A. C., Riker, A. J., Allen, T. C., and Burris, R. H., *Am. J. Botany*, **38**, 550 (1951)
137. Weintraub, R. L., and Norman, A. G., *Econ. Botany*, **3**, 289 (1949)
138. Went, F. W., *Rec. trav. botan. néerland.*, **25**, 1 (1928)
139. Went, F. W., *Arch. Biochem.*, **20**, 131 (1949)
140. Whiting, A. G., and Murray, M. A., *Botan. Gaz.*, **109**, 447 (1948)
141. Wildman, S. G., and Bonner, J., *Am. J. Bot.*, **35**, 740 (1948)
142. Wildman, S. G., Ferri, M., and Bonner, J., *Am. J. Botany*, **33**, 23 (1946)
143. Wilske, C., and Burström, H., *Physiol. Plantarum*, **3**, 58 (1950)
144. Zimmerman, P. W., *Torreya*, **43**, 89 (1943)
145. Zimmerman, P. W., and Hitchcock, A. E., *Contrib. Boyce Thompson Inst.*, **12**, 491 (1942)
146. Zimmerman, P. W., and Hitchcock, A. E., *Contrib. Boyce Thompson Inst.*, **15**, 353 (1949)
147. Zimmerman, P. W., and Hitchcock, A. E., *Contrib. Boyce Thompson Inst.*, **16**, 209 (1951)

AGRICULTURAL APPLICATION OF GROWTH REGULATORS AND THEIR PHYSIOLOGICAL BASIS¹

By J. VAN OVERBEEK

Shell Oil Company, Agricultural Laboratory, Modesto, California

ECONOMIC ASPECTS

Eight years ago when the writer prepared a review on auxins for the *Annual Review of Biochemistry* (173), there were sufficient practical applications for these compounds to warrant inclusion of a section on *Practical Aspects*. At that time the prevention of preharvest drop of apples by naphthaleneacetic acid was the most significant use from an economic point of view. Supplementing pollination for fruit set in tomatoes, preventing sprouting of potatoes in storage, and inducing root formation on cuttings were three other effects of auxins which had come into use in the early forties.

Today, these older uses have been expanded and newer ones have been found. The use of the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), has been so widely accepted that it has become a major agricultural chemical. It is being produced in this country in quantities of 28 million pounds annually (4,170), a figure approaching that of the insecticides 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) (DDT) and benzenehexachloride. Weed control is the principal use for 2,4-D in the United States as well as elsewhere. Thus in Canada, which is reported (4) to produce only three organic pest control products (DDT, 2,4-D, and Parathion), 3.5 million dollars worth of 2,4-D was sold in 1949. In that year the three provinces of Manitoba, Saskatchewan, and Alberta treated over 8 million acres, a figure which increased to nearly 14 million acres the following year (152).

The following figures will serve as an example of the benefit of this extensive use of synthetic auxin for weed control. In these three Canadian provinces in 1949 the 8 million acres of grain sprayed with 2,4-D produced an estimated increase of from three to five bushels per acre. This gain amounts to "an additional yield of from 35 to 40 million bushels of grain on the same acreage simply by the application of herbicidal chemicals" (41).

The wide acceptance of 2,4-D as an agricultural chemical is due to several factors. (a) It is highly effective in low dosages; less than one pound per acre has become standard practice (3). (b) Concentrates have become available that have made it possible to apply 2,4-D by plane in quantities as low as one gallon per acre. Airplane application predominates in the West and Southwest of the United States, while in Canada and in the Middle West of the United States application by ground rig still is of major impor-

¹ The survey of the literature pertaining to this review was concluded in November, 1951.

tance (152). (c) 2,4-D costs at present less than 70 cents per pound. (d) It has a negligible mammalian toxicity (14, 68).

Brush control with synthetic auxins, mainly 2,4-D and the related 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), is another new phase which has become successful. In Oklahoma, control of the sand sage brush (*Artemisia filifolia*) increased beef production two to four times on the same acreage, if it was followed by proper reseeding (136). In the Southwest, a similar procedure has been estimated to increase range capacity by a potential 500 million pounds of beef annually (136).

Changing the pattern of crop production is another use for the synthetic auxins. The most striking example is in pineapple culture, where crop control has become so advanced that the date of harvest can be accurately regulated, thus greatly reducing the peak-season rush. This control is accomplished by a single application of 25 gm. of naphthaleneacetic acid per acre at a total cost of approximately five dollars (176). Synthetic auxins also make it possible to extend the harvest season of field tomatoes (197).

In addition to serving as valuable aides themselves in crop production, synthetic auxins have greatly stimulated development of herbicides of the non-auxin type (136) and that of other agricultural chemicals, such as the flower and fruit thinning agents in the apple industry.

PHYSIOLOGICAL ASPECTS

Although the majority of the agricultural applications of auxins have been entirely empirical, it seems logical to assume that in the future a more rational approach will be made based on an understanding of the physiological principles involved. The main purpose of this review is to arrange the multitude of recent publications in such a fashion that their mosaic forms a coherent picture. This attempt seems especially worth-while for students of the agricultural sciences. It is gratifying to note that while in the past, instruction in agronomy rested primarily on practical facts, recently a trend has become apparent in which students are first being taught the fundamental principles (2).

Several books have appeared in recent years which have greatly helped record progress in the auxin field. For nearly ten years Went & Thimann's classical *Phytohormones* (191), published in 1937, was the only book in the field. This was followed in 1947 by two books dealing with practical aspects of auxins: *Growth Regulators* by Mitchell & Marth (102), and *Hormones and Horticulture* by Avery & Johnson (8). In 1948, two extensive chapters by Thimann in *The Hormones* (162) successfully covered the advances in knowledge in this fundamental field in the decade following the publication of *Phytohormones*. In 1950, the writer published a chapter in *Agricultural Chemistry* (175) written for the specific purpose of acquainting the student in agricultural sciences with the fundamental principles of auxin physiology. In the same year, Bonner devoted a chapter to auxins in his *Plant Biochemistry* (23). A most significant collection of publications dealing with

various phases of auxin activity, as well as that of other growth substances, has been gathered by Skoog, in celebration of the hundredth anniversary of the founding of the University of Wisconsin (145).

The primary auxin reaction.—In order to comprehend the functions of synthetic auxins in agriculture, it is first necessary to come to some sort of understanding of the action of indoleacetic acid, now widely regarded as a principal native auxin. The writer assumes with Bonner (21, 23) that indoleacetic acid affects growth and respiration through the metabolism of phosphate. Phosphate metabolism has been demonstrated to be of utmost importance for the transfer of energy in the organism (97). It is clear, therefore, that if the hormone, indoleacetic acid, can regulate phosphate metabolism, it is in a position to regulate the flow of energy to various other reactions in the plant.² The plant is not alone in such a mechanism, as it has been demonstrated by Cori that in the intact animal, epinephrine, the hormone of the fear syndrome, causes its effects through regulatory action on a phase of phosphate metabolism (30). Phosphate bond energy also serves for the synthesis of acetylcholine in the recovery period of the nerve (97) and the organic phosphorus insecticides owe their effectiveness to their action on the acetylcholine metabolism. It is not surprising, therefore, to find reports (56, 95) which show that conditions can exist wherein organic phosphorus insecticides, such as tetraethylpyrophosphate, and synthetic auxins, such as 2,4-dichlorophenoxyacetic acid, cause similar metabolic and morphological responses in plants.

Auxin and the organic acid metabolism.—The reader will surmise that selection of the phosphate metabolism as the site of primary auxin action is rather a matter of personal preference. Even though evidence in favor of this stand is good, it is still not conclusive. Consequently, other authors have taken a different view. Thus, Thimann sees the role of auxin as that of a protector of an enzyme in the organic acid metabolism against a natural inhibitor (163). Although there is no doubt that there is an intimate connection between auxin and the organic acid metabolism (108, 162, 167), the writer prefers, with Bonner (19), to look upon the organic acid metabolism as a mechanism which provides a substrate for auxin action. According to this view the organic acid metabolism, together with the "endogenous respiration," generates high energy phosphate, the transfer or utilization of which is regulated by auxin.

Thimann and his associates have shown that auxin will only increase respiration if acids of the organic acid cycle are present in sufficient quantities. In other words, conditions exist under which organic acids limit the rate of the auxin reaction. With this fact in mind it is not surprising to find that environmental conditions which lead to increased organic acid production will often cause effects that resemble those caused by auxin. Thus, it has

² A mechanism showing a direct link between auxin and phosphate metabolism has recently been proposed by Rhoades & Ashworth (131a).

been shown that darkness and a drop in temperature result in a substantial increase in organic acids in plants (128, 129, 139, 151). It is also known that a drop in night temperature causes pineapples to flower (179) and that flowering in this plant can be achieved by synthetic auxins without the drop in night temperature (176). It has recently been proven, through research in greenhouses with strictly controlled environmental growth conditions, that cucurbits (118), as well as tomatoes (124), set fruit without auxin and without pollination in response to a drop in the night temperature. The reader will recall that auxins also will induce parthenocarpic fruit set (55).

Auxin and enzyme reactions.—Striking changes in enzyme activities are often found in tissues treated with auxin. This auxin effect must be largely indirect since so far no such activities have been observed in isolated enzyme systems. These have been discussed by Bonner in this volume. These enzyme changes are important because they are the basis of the physiological effects caused by auxins. Not always, however, is a change in activity of one specific enzyme responsible for a specific auxin response. It would seem that often a change in balance between the activities of the various enzymes is responsible for the auxin responses which one observes. The fundamental effect is then a tendency to a changed type of respiration (148) or a changed type of metabolism (181). This fact was well demonstrated by Teubner (161), who showed that, in tomato ovules, addition of low concentrations of auxin stimulated the malate and fumarate respiration, but suppressed the glutamate and succinate respiration. The synthetic auxin, *p*-chlorophenoxyacetic acid, has a similar effect in cabbage roots, causing the bypassing of normal metabolic intermediates such as succinates (75). The changed metabolism induced by auxin shows up in numerous other ways (181). One may be mentioned specifically as it suggests an explanation of the so-called "formative" effect of substituted phenoxyacetic acids. This formative effect is in reality but a "frenching" of the foliage. Frenching of tobacco is caused by abnormal accumulations of free amino acids (153). Perhaps it is significant that comparable effects on the amino acid level in plants are caused by 2,4-D treatments (125).

Even though a direct action of auxin on dehydrogenases and oxidases seems unlikely at present (98, 148, 185), the indirect effects may well link auxin to important physiological processes. Thus, Newcomb discovered a link between auxin and ascorbic acid oxidase (114). He presented considerable evidence that this cyanide sensitive copper protein occurs at the surface of the cell and that its activity is positively and causally related to cell elongation.

An inverse correlation was found between growth rate and catalase activity in plant tissue cultures (47). By analogy, dormant potatoes were found to have a higher catalase content than active ones (168). Since catalase and the peroxidase member of the indoleacetic acid oxidase complex (46) both use the same substrate (H_2O_2), it would seem that in dormant potatoes the high catalase activity protects the hormone, indoleacetic acid, from de-

struction by the indoleacetic acid oxidase system. Thus, the maintenance of a high auxin content is assured which in turn prevents sprouting. Dormancy of potatoes can be prolonged by artificially maintaining a high auxin level (see below). An increased catalase activity has also been reported by Hsiang (73) as the earliest metabolic change following pollination. This activity may well constitute an auxin-sparing reaction and be one of the phases leading to increased auxin activity in the pollinated ovary.

Auxins and pectins.—A further auxin-induced change in enzyme activity of importance for the interpretation of auxin action has been reported by Neeley *et al.* (112), who showed that pectin-methylesterase activity is increased in tissue treated with the synthetic auxin, 2,4-D. This increased pectin enzyme activity is considered to be associated with the observed breakdown of the protopectin in cell walls of plants treated with 2,4-D. The primary cell wall contains protopectin which is water insoluble but which can hydrolyze into pectic acid (free of methyl groups) and pectinic acid (partly methylated) (78). This observation becomes significant (112) if one dissociates oneself from the prevailing view [Bonner (23), Frey-Wyssling (44a)] that auxins control cell elongation indirectly through the cellulose of the primary wall. Twelve years ago the writer advanced the idea (172) that pectins and related compounds, rather than cellulose, strengthen the young growing cell in the longitudinal direction, and that the auxin-induced plasticity of the primary cell wall could be explained by the action on these compounds rather than on cellulose. Audus also sees the action of auxin on elongation as a swelling of intermicellary substance (6). Recently, Kerr (78) also came to the conclusion that the pectins constitute the continuous phase in the primary cell wall and that the properties of cell-wall behavior can be explained on this basis and not on the basis of cellulose. It would appear, therefore, that auxin, through stimulation of the pectin-methylesterase, initiates the breakdown of protopectins which in turn results in a reduced tensile strength of the young primary wall. The latter is subsequently stretched by the turgor pressure of the cell.

This concept of cell elongation suggests an explanation of the calcium-sparing action of auxins observed by Struckmeyer (160). The increased breakdown of protopectin in the plants treated with auxin keeps more calcium mobile because less of it is tied up in the primary wall as calcium bonds between the carboxyl groups of the chains of anhydrogalacturonic acid of the protopectin. Permanent weakening of the cell does not take place because of a disposition of cellulose in the secondary wall. Many readers will also recall Eaton's work of over a decade ago (39) in which he demonstrated that auxin will to some extent alleviate the symptoms of boron deficiency. As with calcium, a tie-up between pectins and boron has occasionally been proposed (70).

Another set of phenomena which might well be explained through auxin-pectin relationships is the so-called June drop and the thinning of young apple fruits with synthetic auxins such as naphthaleneacetic acid. In these

cases, a rapidly developing fruit puts such a strain on the carbohydrate supply that the elongating cell walls at the base of the fruit stalk cannot acquire strengthening cellulose deposits fast enough. The result is that the primary cell wall, weakened by the above described action of auxin, does not have tensile strength enough to support the fruits which rapidly gain in weight. It will be evident that this type of fruit drop is not considered a true abscission.

True abscission involves the breakdown of the middle lamella (18, 23). This layer consists of water-insoluble calcium pectate, which, in contrast to the protopectin of the primary wall, is not or is very little methylated (78a). The physiological breakdown of the pectin of the middle lamella may be enzymatic (137, 147) or it may not be (78a). If the breakdown is enzymatic, depolymerase, which breaks the pectin between the chains, may be involved, rather than pectin-polygalacturonase, which breaks the chains themselves

TABLE I
SUGGESTED RELATIONS BETWEEN AUXIN AND PECTIN

Basic Mechanism	Location	Phenomenon
Decomposition of protopectin (promoted by auxin through pectin-methylesterase)	Primary wall of aerial organs	Elongation, June drop of fruit
Decomposition of calcium pectate (inhibited by auxin)	Middle lamella	Abscission, cracking of fruit, physiological soft rot
	Root cell wall	Elongation

(78a). Regardless of the mechanism involved, auxins inhibit abscission (23, 48), presumably through inhibition of the physiological breakdown of the calcium pectate of the middle lamella. This will be discussed in more detail below in the section dealing with abscission of fruits. One suggestion may be made at this point. It appears that the pectin of the root cell wall is calcium pectate (78a). In this respect the root cell wall resembles the middle lamella rather than the primary wall of aerial organs. It may be significant that also in its response to auxins the root cell wall resembles the middle lamella, rather than the primary wall of aerial organs. Including the data on fruit physiology the relations shown in Table I are suggested.

Auxin and virus.—Another fascinating aspect of the auxin metabolism is the observation made by Wildman (195) that in tobacco leaves virus protein is formed at the expense of the normal cytoplasmic protein. The latter is a nucleoprotein with phosphatase activity and is the major auxin protein (23). It is also known that synthetic auxins such as 2,4-D and methoxone inhibit virus multiplication strongly (88), especially when virus inoculation and auxin treatment are given at the same time. In tobacco tissue cultures,

1 p.p.m. of naphthaleneacetic acid decreased the virus concentration by one-third (81). The writer suggests as an explanation that when the auxin protein has naphthaleneacetic acid or some of the other synthetic auxins for a haptenic group instead of the native indoleacetic acid, such a protein becomes less susceptible for transformation into a virus protein.

Auxin and molecular structure.—An up-to-date review of the relation between auxin activity and molecular configuration has been presented by Thimann (163). It appears that generally a molecule has primary auxin activity when (a) it has a ring system which is active interfacially and (b) it carries an acidic polar group, preferably a carboxyl group which has an average spatial position over or perpendicular to the plane of the ring. Several active compounds have been found which deviate from this basic structure. Thus, it has been thought that esters, amides, and amines of auxins are active per se and do not have to be broken down into the parent acid for auxin activity (96, 163). Apparently the carbonyl group must be retained on the side chain, while the hydroxyl group may be substituted (96).

In the chlorinated phenoxyacetic acid group it has been shown that chlorine in the 4-position is essential for activity, which is lost when chlorine is replaced by amino, nitro, and other groups. The chlorine in the 2-position, by contrast, is of secondary importance and may be replaced by methyl without serious loss of activity (96). When both *ortho* positions are chlorinated in the phenoxyacetic acids, activity is lost completely (84, 138), which has led Muir *et al.* (60, 104) to the conclusion that these auxins react with a —SH group of the plant substrate (60a) through the unsubstituted *ortho* position. In addition, Leaper & Bishop (84) showed that for high physiological activity of the chlorophenoxyacetic acids there must be two unsubstituted positions in the benzene ring *para* to each other. Thus, 2,4,5-trichlorophenoxyacetic acid is a highly active auxin, while 2,3,5-trichlorophenoxyacetic acid is inactive. An alternative explanation is possible, however, for the loss of auxin activity when both *ortho* positions are substituted. If one accepts the common assumption that the ring of indoleacetic acid attaches itself at a specific locus on a cytoplasmic protein, it follows that this locus has an outline ("lock") corresponding to that of the indole nucleus ("key"). Synthetic auxins likewise will have to fit this "lock." This outline of the locus has two prominent features: (a) an indentation where the NH of the indole nucleus fits; and (b) a bulge corresponding to the narrow neck or space between carbon atoms three and four of the indole nucleus. The chlorine atom in the 2-position in the phenoxyacetic acids fills the space normally occupied by the NH of indoleacetic acid. Since this chlorine atom occupies a space equivalent to that of a methyl group, it is understandable why methyl and chlorine at the 2-position are interchangeable in the phenoxyacetic acids. If in these acids, the other *ortho* position (the 6-position) were also occupied, it would prevent the ring from fitting into the locus because of the narrow neck referred to under (b) above. A study of Hirschfelder models will at once verify this statement.

Another item which is creating controversial opinions is the discovery

that a number of halogenated benzoic acids and their aldehydes cause strong auxin reactions (11, 105, 163, 203). These benzoic acids have the carboxyl group on the ring and therefore do not fulfill the three basic requirements for auxin activity. It was found (17), however, that these acids do not act like typical auxins because they do not cause a growth response in corn coleoptiles, a tissue which responds well to typical auxins such as indoleacetic acid, naphthaleneacetic acid, 2,4-D, etc. Since corn coleoptiles were also found to be low in enzyme activity, it is suggested that the chlorinated benzoic acids and aldehydes have to be converted into auxins by the plant in order to induce growth responses. The reverse of a similar reaction is the oxidation of indoleacetic acid by the indoleacetic acid oxidase system to indolealdehyde (23). The conversion of indoleacetaldehyde to indoleacetic acid and the conversion of other auxin aldehydes into their respective acids, also occurs readily in plant tissues (5, 53, 82, 83). In addition, studies with radioactive tracers have shown that synthetic auxins are broken down inside the plant (26, 71, 189), and sometimes at a rapid rate. Enzyme reactions are reversible, although not necessarily by the same path. Recently, Hansch *et al.* (60a) have directly demonstrated that in the plant, chlorine is released from 2,6- and 2,4-dichlorobenzoic acids and that this release is correlated with growth. It would seem, therefore, that there is enough justification to warrant the assumption that chlorinated benzoic acids and aldehydes undergo changes in the plant which turn them into compounds which meet the three basic requirements for auxin activity. Therefore, rather than regarding these benzoic acids as an exception to the rule, the writer is more inclined to consider these compounds as auxin precursors than as auxins.

Auxin activity and pH.—The activity of auxins depends upon the pH of the medium, a fact recognized for a long time (191). It appears that auxin activity is correlated with the concentration of the undissociated auxin acid rather than with the total auxin concentration. This same concept has been expressed in a variety of ways (99, 133, 140, 141). If one asks what this correlation means in physiological terms, the answer may well lie in the fact that acids occur in the undissociated state in the lipid phases of the living matter. It is possible, therefore, that the concentration of auxin in these lipid phases (plasma membrane, lipoproteins, cuticle, etc.) is the "limiting factor" and therefore determines the activity of auxin in the tissue. Veldstra (171) has pointed out the significance of the lipophilic properties of the auxin molecule, while Crafts (31) showed that such properties promote penetration of herbicidal compounds through the cuticle of leaves. This view would also explain the observation that 2,4-D applied in the acid form is physiologically more active (57) and is translocated more readily (100) in the plant than when applied in the salt form.

Inhibition of auxin activity.—The level of the hormone, indoleacetic acid, in the plant is maintained by a balance between production and destruction. The latter is brought about by an indoleacetic acid oxidase system. This is composed of two enzymes: (a) a flavoprotein which produces H_2O_2 , and (b) a

peroxidase which then specifically oxidizes indoleacetic acid (46). Light promotes this method of indoleacetic acid destruction by producing more H_2O_2 , while agents which reduce the amount of H_2O_2 in the plant will tend to inhibit indoleacetic acid destruction. Other synthetic auxins such as naphthaleneacetic acid, 2,4-D, etc., are not attacked by the indoleacetic acid oxidase system, although they are attacked by other enzyme systems. This is one reason for the greater stability in the plant of synthetic auxins other than indoleacetic acid. This greater stability is a reason auxins such as indolebutyric acid, naphthaleneacetic acid, 2,4,5-trichlorophenoxypropionic acid, etc., are used in agricultural practice for growth regulation of all sorts in preference to indoleacetic acid.

Another way in which auxin activity may be inhibited is by using compounds which deviate but slightly from the three basic requirements for auxin activity. These compounds inhibit auxin action competitively. Examples are: 2,4-dichloroaniline (23), α -(1-naphthylmethyl sulfide)-propionic acid (1), and *trans*-cinnamic acid (180).

Bonner (23) and Thimann (163) have pointed out that the growth process involves enzymes which are highly sensitive to sulfhydryl reagents. Accordingly, a number of such reagents have been shown to interfere with the auxin response. Some of these are: iodoacetate, *p*-chloromercuribenzoate and phenylmercuric chloride (165), protoanemonin, coumarin and other lactones (52, 166), and maleic hydrazide (74, 111, 201).

WEED CONTROL

The great upsurge in chemical weed control began suddenly, less than ten years ago, when it was found that some of the synthetic auxins had selective herbicidal properties and that these compounds could be manufactured cheaply and in large quantities. This great progress was followed by a stream of some 2,500 publications on weed control annually (136). No attempt will be made to review these, but instead a brief discussion will be given of the physiological basis of the phytotoxic action of the auxins. The principles of selective toxicity of herbicides have been skillfully reviewed by Blackman (13). General summaries have also appeared in previous volumes of the *Annual Review of Plant Physiology* (14, 121), while extensive sections of the book *Plant Hormones in Agriculture* (169) are also devoted to this topic.

Among the many suggestions which have been made to explain the action of 2,4-D are the following: interference with the native auxin, indoleacetic acid (51, 144); interference with the phloem (38, 149); and interference with numerous physiological and enzymatic processes (14, 121, 181). Selective action has been thought to depend upon cuticle thickness (69).

Although the principal auxin herbicides of commerce are phenoxyacetic acids (2,4-dichlorophenoxyacetic acid [2,4-D]; 2-methyl, 4-chlorophenoxyacetic acid [Methoxone]; and 2,4,5-trichlorophenoxyacetic acid [2,4,5-T]), the writer and his associates have pointed out (181) that potentially all auxins have herbicidal properties. In fact, the first demonstration of the

selective herbicidal properties of an auxin was made with naphthaleneacetic acid in England in 1940 (12). When the activities of the various auxins were compared on the basis of undissociated acids (see above), the chlorinated phenoxyacids were found to be much more active than auxins such as naphthaleneacetic acid and indoleacetic acid. This difference in activity was not constant, but increased progressively with concentration. Thus, it was concluded (181) that in the range of herbicidal effects, molecule for molecule 2,4-D may have an activity of one-million times that of indoleacetic acid. It is this great physiological activity of the chlorinated phenoxyacetic acids which sets them apart from the other synthetic auxins and which makes their economical use as herbicides possible. Even if the phenoxyacids had been only 10 times less active (range of naphthoxyacetic acid) than they actually are, they would not have been economically as successful as 2,4-D is today. A drop in activity by a factor of 10 means a tenfold increase in cost of chemical per acre, a tenfold increase in shipping and storage cost, and a reduction by a factor of 10 in the number of acres a plane can spray without landing.

What exactly confers such a high auxin activity to the 2,4-D molecule is not too well understood, but the following facts appear pertinent: (a) 2,4-D is not attacked by the oxidase which rapidly destroys the native auxin indoleacetic acid in the plant; (b) chlorination of the ring increases lipoid solubility (Veldstra); (c) introduction of electro negative atoms (such as chlorine) or electro negative groups in the benzene ring enhance reactivity, especially when the substitution is made in the *para* position (Hansch); and (d) chlorine (or methyl) in the *ortho* position tends to keep the side chain in the active position (viz. over or perpendicular to the ring) by steric hindrance.

The toxic action of high concentrations of auxins has been discussed elsewhere (174, 181) and is almost certainly due to the auxin-induced, changed type of respiration or metabolism discussed in an earlier section. Death of the plant tissue is thus viewed, not as a direct result of 2,4-D, but as the result of abnormal concentrations of metabolites which usually are present in smaller quantities. As an example of such metabolites, an increase in coumarin derivatives was cited as many of these are known to be highly toxic (see above) and, in addition, display selective properties (58). An abnormal accumulation of such metabolites would therefore explain both the toxicity and selectivity of high concentrations of auxins such as are used in herbicides (181).

The fate of 2,4-D in the soil has been discussed elsewhere (121, 177). Three phases can be distinguished: (a) an immediate initial adsorption on to the soil colloids differing markedly among the various soil types (25, 107); (b) a lag period during which little material disappears; and (c) a final phase of rapid and complete bacterial decomposition (7). Pretreatment of the soil with 2,4-D speeds up bacterial decomposition which results from the building up of microorganisms (116). These effects are quite specific; thus 2,4-D

enriched soils break down added 2,4-D and also Methoxone, but not 2,4,5-T (7).

CONTROL OF FRUIT CROPS

Auxins and flower formation.—Flower initiation in the pineapple by naphthaleneacetic acid is at present the only major use of auxins in this phase of growth and development. Recently the way has been paved for another economically significant application. Low concentrations (5 p.p.m.) of 2,4-D (127) retard flowering (bolting) in the sugar beet. This action would insure a considerable spreading out of the harvest season, and, in addition, facilitate machine harvesting in years when, because of climatological conditions, bolting is apt to become troublesome. Auxin sprays, however, do not increase the yield or sugar content of beets (113).

As far as the physiology is concerned, auxin appears to affect flower formation in three ways: promotion, inhibition, and a shift toward the female type of flower. There is little doubt that in the pineapple, auxin is involved in the process of flower formation. This activity has been discussed recently (176). Clark & Wittwer (27) have presented evidence that auxin must be involved also in the flower formation of celery. They believe that the auxin theory of flower induction in the pineapple fits the data for celery better than the florigen theory of Cailahjan. However, a compromise may be in sight as Hamner (59) has recently suggested that in certain plants auxin may be a required factor for the formation of florigen. This view acquires added interest in the light of a recent suggestion by Bonner (22) that florigen may be a protein with virus-like properties. Wildman (195) has found a definite relation between auxin protein and virus protein.

In the majority of plants, auxins bring about a delay in flowering (132, 183). Anti-auxins have been shown to promote flowering under suitable experimental conditions (20, 122). Bonner has shown that auxin is capable of interrupting the dark reaction which takes place during the long nights required for flowering of short-day plants (22). It is well known that this same dark reaction is also interrupted by a brief exposure of the leaf to light. Similarly, Wittwer (196) demonstrated that 1 p.p.m. 2,4-D inhibits flowering in lettuce induced by vernalization. It is essential that auxin be applied during the vernalization period. Auxin applied before or after that period has little effect.

Another type of physiological reaction by which auxin can inhibit flower formation is the process of bud inhibition. Reece, Furr & Cooper (130) have shown that flower induction will take place only when nonpreformed axillary buds (as in the mango and other tropical plants) are undergoing cell division. Thus, if bud development is suppressed by auxin action, no flowers are formed. Similarly, enzyme poisons which inhibit growth thereby will prevent the induction of flowers. This process is exemplified by reports (76, 194) which show that maleic hydrazide inhibits flower formation.

Nitsch (119) showed that in cucurbits, treatment with naphthaleneacetic

acid would speed up the appearance of female flowers. Normally these plants produce male flowers when young and with increasing age produce respectively, hermaphroditic, female, and parthenocarpic female flowers. Naphthaleneacetic acid makes female flowers appear earlier than they would without auxin treatment. A drop in night temperature accomplishes the same result and suggests to the writer that possibly organic acid metabolism may be involved. In olives, abundant production of staminate flowers and only a few perfect flowers is sometimes a problem. Girdling causes a shift to production of more flowers of the perfect type (63). Perhaps auxin sprays also might bring about this shift.

Auxin and fruit set.—Crop yields of tomatoes in the field may be substantially increased by auxin sprays. In England, good results were obtained by spraying a mixture of 10 p.p.m. of *p*-chlorophenoxyacetic acid and 40 p.p.m. β -naphthoxyacetic acid applied to the bottom trusses (186). In the United States, a somewhat similar mixture of these two compounds applied to the whole tomato plant in the field has given good results. It increased the early yield without loss to the total yield for the whole season (143). Auxin sprays are used specifically when night temperatures are below 59°F. (200). In greenhouses, also, the mixture of the two synthetic auxins is used and has given the largest yields when applied four days after anthesis (65). This outcome is explained by the presence of seeds which are natural centers of auxin production in these fruits. It has also been reported that a combination of *p*-chlorophenoxyacetic acid and indolebutyric acid applied to the base of pollinated flowers increased the number of successful crosses in lima bean breeding (135). The writer has also heard of farmers who use naphthaleneacetic acid sprays at full bloom in order to increase seed set in alfalfa. In this connection it may be well to remember that poor seed production may not always be physiological and that sometimes insecticides interfere with bees and other beneficial insects necessary to pollinate alfalfa, onions, and other seed crops (79).

Blondeau & Crane succeeded in replacing wasp pollination of *Calimyrna* figs by a spray of 60 p.p.m. *p*-chlorophenoxyacetic acid (16, 34). This accomplishment has led to a new commercial product in which the auxin-induced seedless figs are processed in the juice of regular figs for flavor (126). Another fruit in which auxins are used to induce set is the Black Corinth grape (*Zante currant*). Concentrations between 25 and 50 p.p.m. of *p*-chlorophenoxyacetic acid applied at full bloom, directly to the clusters, caused development of large clusters of fruit without the customary annual girdling of the vines (29, 188). To the writer's knowledge this process has not yet gone into commercial operation.

Increased set of pomaceous fruit is difficult, but has been achieved in Bartlett pears (36) by sprays of 2,4,5-trichlorophenoxypropionic acid, and in other pears and one apple variety by repeated applications of α -(2-naphthoxy)propionic acid (123). In pomaceous fruits, however, there is generally much more use for a fruit thinning spray (see section on abscission) than for sprays which further increase the number of fruits per tree.

The physiological basis of auxin-induced fruit set is to make available to the fruit an adequate supply of auxin during the early stages of development (123). Normally this result is brought about by pollination and fertilization, two distinct phases in the auxin supply picture. Where this natural supply of auxin is inadequate, it may be corrected by the synthetic auxin sprays, which induce changes in the ovary duplicating those normally brought about by pollination (73). In addition to the compounds already mentioned in this section, Zimmermann has reported that 2,5-dichlorobenzoic acid is especially active for inducing parthenocarpic fruit (202). If this compound is indeed converted into an auxin (see above), one would expect that its application would cause a gradual release of auxin, thus more closely approaching the natural process.

Auxin and fruit growth.—As auxin prevents abscission, it has often been thought that after pollination causes an increase in the auxin content of the ovary, the prevention of flower abscission automatically causes the young fruit to grow. Osborne & Wain (123) pointed out that auxins delay blossom abscission, but that this effect is not necessarily related to auxin-induced fruitlet development. Nitsch (120) also showed with experiments on gherkins and tomatoes that prevention of abscission is a prerequisite to ovary development but not its cause. The latter is undoubtedly the auxin produced in the seeds. This fact has been suspected following the early auxin determinations of Gustafson. Recently Nitsch (117) has provided conclusive evidence with experiments on the strawberry. When, in this fruit, the achenes ("seeds") are removed, growth stops, regardless of the stage of development. Analysis showed that the fertilized achenes produced increasing amounts of auxin up to 12 days after pollination. Fruits from which the achenes had been removed resumed growth after application of synthetic auxin. Because of the external location of the auxin production, the outer parts of the strawberry fruit grow faster than the inner. In cucurbits, with centrally located ovules, the inner part grows more rapidly than the outer (the reason why watermelons break so easily).

Evidence points to the conclusion that in a number of fruits auxin limits growth during certain phases of development. Addition of synthetic auxin will increase the growth rate of these fruits. In 1948 Blondeau & Crane (15, 32, 33) were the first to show that application of the synthetic auxin, 2,4,5-trichlorophenoxyacetic acid, will shorten the normal ripening period of the Calimyrna fig so drastically that the average 120 day period is cut in half. Later, a similar treatment was found to shorten the ripening cycle of apples and peaches by a month (61, 93, 94). These observations are, however, more of theoretical than of practical interest at present. In citrus, by contrast, acceleration of the growth rate by auxins has found practical use. Application of a few parts per million of 2,4-D, six to eight weeks prior to bloom, increases the size of Washington navel oranges at harvest time (158). Similar results have been obtained with limes (42).

Auxin and abscission.—In fruit trees three types of drop occur (184): (a) a first wave of unpollinated or unfertilized blossoms; (b) a second wave,

known as the June drop, when young fruits abscise; and (c) the pre-harvest drop. It would appear that (a) and (c) are true abscission and the result of auxin deficiency: consequently, they can be corrected by applications of synthetic auxin (48, 89). The writer considers (b) not a true abscission, but rather an exaggerated cell elongation, brought about by high auxin content and a slow rate of cell wall formation (see section on *Auxin and Pectins*). Hence, applications of auxin will promote type (b) abscission, which has found practical application in the thinning of young apple fruit (106).

Thinning of flowers and young fruit is a required operation designed to increase fruit size (as in peaches) and also eliminate the biennial bearing habit (as in apples). While in peaches not much progress has been made with chemical thinning, it is rapidly replacing hand thinning in the apple culture. Batjer & Hoffman have summarized experience with it (10). Two types of chemicals are being used on apples: dinitrophenol derivatives and auxins. The dinitro compounds act as pollenicides and thus induce abscission of type (a). They have to be applied at the blossom stage and require careful timing. Auxins, on the other hand, are applied a month after full bloom and do not require accurate timing. Auxin thinning, therefore, has the advantage that it is done at a time when losses caused by frost have become unlikely. It has the disadvantage, however, that at the time thinning is done much fruit growth has taken place already and for this reason auxin thinning is not so effective as dinitro thinning for the elimination of biennial bearing. Growers in the western United States seem to prefer dinitro thinning, while apple growers in the central and eastern part of the country prefer to use auxin thinning. Naphthaleneacetic acid at concentrations ranging from 5 to 60 p.p.m. are used for thinning (10, 66), and it is important to note that such sprays tend to eliminate the type of fruit which is likely to develop into low grade apples at harvest (10). Auxin thinning has not been successful on peaches and prunes (87) but 150 p.p.m. of naphthaleneacetic acid gave the desired grade of thinning of young olive fruits (64).

It has been known for 12 years that raising the auxin concentration in a plant prevents abscission (48). At present, control of preharvest drop by applications of synthetic auxins has become a standard orchard practice for apples and pears (48). Conversely, true abscission is preceded by a drop in the auxin level of the plant (142). Abscission usually takes place at the base of the fruit stalk, but whether the "abscission layer" is causally related to abscission or is merely a protective layer has been subject to argument (50), at least for foliar abscission. It is a fact, however, that the cell walls in the abscission layer have a middle lamella of calcium pectate (44) which differs by its lack of methylation from the pectins of the primary wall (78). Abscission is, therefore, due to middle lamella breakdown, possibly enzymatic (18). Softening of fruit accompanying ripening has also been shown to be due to enzymatic dissolution of the middle lamella (147). In tomatoes, pectin-depolymerase is involved in this process (78a). At the end of the growing season the plants' respiratory rates decline (115) and with them the auxin levels. This decline in turn leads to increased breakdown of the calcium pec-

tate of the middle lamella, which again causes both the softening of the fruit and its abscission from the tree. Conversely, a timely increase in the plant's auxin level through synthetic auxins will prevent physiological reactions associated with middle lamella breakdown. Among these reactions appears to be, in addition to the ones already mentioned, the cracking of cherry fruits. Both calcium and auxin will prevent cracking (9, 24), so that strengthening of the calcium pectate of the middle lamella of the fruit's superficial tissues appears a logical explanation. It is possible that toughening of the fruit skin by preventing middle lamella breakdown will turn out to be a general function of auxin. Blossom end rot of tomato fruit, a physiological rot brought about by drought, can be improved by auxin applications (197). Here, also a breakdown of the calcium pectate of the middle lamella of the fruit cells is involved which could be checked by timely application of auxin. For a summary of auxin-pectin relations the reader is referred to Table I.

Another phase of ripening fruit, the conversion of starch to sugars, is promoted by auxin, but at relatively high concentrations (101). This phase is in all probability associated with the auxin-induced improvement in fruit color occasionally reported (9, 24).

The synthetic auxin most commonly used for the control of preharvest drop in apples is naphthaleneacetic acid at the rate of 48 gm. per acre. It never performed well on Winesap apples where 2,4-dichlorophenoxyacetic acid had to be used. Recently 2,4,5-trichlorophenoxypropionic acid has been introduced for the control of preharvest drop (9, 40). This new material seems more persistent than naphthaleneacetic acid and can be applied four weeks earlier, while still retaining full protective effect at harvest time. Preharvest control with auxins is also practiced in pears, especially those which are harvested for canning at a relatively advanced state of maturity (48).

Since 1947, satisfactory control of preharvest drop of citrus fruit has also been achieved in California by applications of 2,4-D as low as 4 p.p.m. in a drenching spray (159). Good results have also been obtained in Australia (45). Reports from Florida have been less outstanding (48, 134). Spraying with 2,4-D is becoming standard practice (159). Treatments with 2,4,5-trichlorophenoxyacetic acid have also come into use and have been found more effective than 2,4-D (155, 157). In lemons, these auxins prolong storage life by preventing abscission from the cut fruit stem and thus reduce entrance of fungi causing black button and internal *Alternaria* decay (156). With Florida limes, similar results have been obtained (49).

Synthetic auxins have been used to control abscission in several other instances. Leaf abscission was prevented in stored cauliflower (72), winter-grown begonia flowers (187), petals of oriental cherries, and bracts of dogwood (192). Perhaps the oldest treatment to prevent preharvest drop is the daubing of olive oil on figs some 10 days before harvest (28). This treatment, as old as history, also speeds ripening, and its results may well be due to auxin or its precursors contained in the oil. Vegetable oils are known for their auxin content and the reader will recall that the first auxin was isolated from corn oil (191).

At the conclusion of this section of auxins and abscission, one further example must be given of the far-reaching economic implications of synthetic auxin treatments. In the Pacific Northwest the climate is suitable for holly, but before the era of synthetic auxins its market was limited to a small radius. The product was so perishable that growers had to wait until a few days before Christmas and rush it to the buyers before the leaves and berries fell off. For several years now, a naphthaleneacetic acid dip has been used which prevents abscission of both leaves and berries. This treatment allows growers to start shipping late in November, thus expanding the marketing radius, and now the Northwest produces most of the nation's holly (160a).

CONTROL OF ORGAN FORMATION AND DEVELOPMENT

Auxin is intimately associated with the formation and development of organs in plants. It has long been recognized as a critical agent in the initiation of root primordia. It is known to inhibit the development of axillary buds. It either promotes or inhibits flower initiation (see above) and experimental data suggest that auxin plays a part in the inception of leaf and bud (193).

Work on isolated tissues grown *in vitro* has added much in recent years to the understanding of the function of auxin in growth and development. It is clear at present that all tissues require auxin for these processes. They acquire it either from the medium (most normal tissues) or from inherent synthesis (most pathogenic tissues) (67). Skoog (146) has demonstrated that a high auxin concentration in tobacco tissue culture favors root formation and prevents bud formation. There is a distinct balance between auxin and other cofactors, however, such as adenine. A high concentration of adenine favors bud formation and inhibits root formation. Another most interesting study is that by Levine (85, 86) which demonstrated the regulatory effect of auxin in combination with other growth factors. Fibrous roots of tobacco growing *in vitro* produced compact nodular structures only in the combined presence of indoleacetic acid and coconut milk. These nodules, when excised and transferred, subsequently developed into a completely differentiated plantlet. Hence, an auxin in the presence of a proper cofactor induced an organ in the root capable of differentiating into a complete plant. The coconut milk factor was discovered as a growth factor for higher plants ten years ago by the writer and his associates (178). This factor, chemically still unidentified, enabled Morel & Wetmore (103) to grow monocot tissue *in vitro* for the first time. It also stimulates growth of other tissues *in vitro* (37), and is especially active as such in the presence of auxin (154).

In the practical line, established applications continue to be used. Rooting of cuttings with auxins is an established nursery practice. One of the largest single uses is perhaps in the vegetative propagation of cacao with a mixture of indolebutyric and naphthaleneacetic acids. In all the West Indian Islands, as well as in West Africa, this use of auxin has become a standard method in the propagation of cacao from cuttings (43). The potential use of

auxin in this field is well exemplified by a recent report (62) considering the planting of 250,000 acres to cacao in Malaya. The planting material has to be propagated vegetatively from high yielding clones both by the budding and the cutting technique. An extensive summary of the many species rooted is available (164), while the fundamental aspects of the process of root formation has also been discussed recently (176). In this discussion, special emphasis was placed on the chemically identified cofactors to auxin. Interesting in this regard is the experiment by Naylor, who showed that 2,4-D does not cause root formation in stems of *Xanthium* subjected to flower-inducing photoperiodic treatment, while the control plants which had not received the photoperiodic treatment produced roots under the influence of the synthetic auxin (110). Hemi-auxins, compounds which resemble auxins without being active by themselves (190), were shown to enhance root formation induced by indoleacetic acid (182). Introduction of cofactors by grafting a bud of a young plant, together with auxin treatment, produced roots for the first time on cuttings of a hard-to-root *Hevea* variety (54).

Auxins still continue to be used as sprout inhibiting agents of stored potatoes. The methyl ester of naphthaleneacetic acid and the methyl, ethyl, butyl, and isopropyl esters of 2,4,5-trichlorophenoxyacetic acid were highly effective as sprout inhibiting agents on stored table potatoes, as well as on cull piles, the latter preventing dissemination of spores of *Phytophthora infestans* (92). In a series of 1-(alkoxymethyl) naphthalenes it was shown that when the alkoxy group is lengthened, the capacity to inhibit sprout formation decreases. The higher homologues such as amyloxy and cyclohexyloxy favored sprout formation (80). Successful attempts have also been made to spray plants in the field to obtain inhibition of sprouting of the stored tubers (91, 150).

Among the compounds other than auxins which have been used to suppress sprouting in potatoes are the following; 2,3,5,6-tetrachloronitrobenzene (90), isopropylphenyl carbamate (131), and maleic hydrazide (77, 198). The latter compound can also be applied on the foliage in the field. Because of its interference with auxin (see above), maleic hydrazide causes an increased number of eyes to grow out, but the total weight of the sprouts was below that of the control. This fact, together with its capacity to inhibit growth of active and of terminal buds (109, 199), places maleic hydrazide as an inhibitor, in a different class from the auxins. It is probable that it owes its inhibitory activity to its properties as a respiratory poison (see above). Inhibitors of this type have the advantage that the weight losses of tubers associated with the auxin inhibitors (90) can be avoided.

LITERATURE CITED

1. Åberg, B., *Physiol. Plantarum*, **3**, 447 (1950)
2. Ahlgren, H. L., *Agronomy J.*, **43**, 367 (1951)
3. Allen, W. W., *Agr. Chemicals*, **6**(5), 45 (1951)
4. Arrington, L. G., *Agr. Chemicals*, **6**(2), 53 (1951)
5. Ashby, W. C., *Botan. Gaz.*, **112**, 237 (1951)

6. Audus, L. J., *Biol. Revs. Cambridge Phil. Soc.*, **24**, 51 (1949)
7. Audus, L. J., *Plant and Soil*, **3**, 170 (1951)
8. Avery, G. S., Jr., Johnson, E. B., Addoms, R. M., and Thompson, B. F., *Hormones and Horticulture* (McGraw-Hill, New York, 326 pp., 1947)
9. Batjer, L. P., *Better Fruit*, **46**(2), 7 (1951)
10. Batjer, L. P., and Hoffman, M. B., *U. S. Dept. Agr. Circular*, No. 867, 1-46 (1951)
11. Bentley, J. A., *Nature*, **165**, 449 (1950)
12. Blackman, G. E., *Nature*, **155**, 497 (1945)
13. Blackman, G. E., *Sci. Progress*, No. 152, 637 (1950)
14. Blackman, G. E., Templeman, W. G., and Halliday, D. J., *Ann. Rev. Plant Physiol.*, **2**, 199 (1951)
15. Blondeau, R., and Crane, J. C., *Science*, **108**, 719 (1948)
16. Blondeau, R., and Crane, J. C., *Plant Physiol.*, **25**, 158 (1950)
17. Blondeau, R., van Overbeek, J., and Horne, V., *Abstracts Am. Soc. Plant Physiol. Meeting*, 11 (Los Angeles, California, 1951)
18. Bonner, J., *Botan. Rev.*, **10**, 475 (1936)
19. Bonner, J., *Am. J. Botany*, **36**, 429 (1949)
20. Bonner, J., *Botan. Gaz.*, **110**, 625 (1949)
21. Bonner, J., *Plant Physiol.*, **25**, 181 (1950)
22. Bonner, J., *Abstracts Am. Soc. Plant Physiol. Meeting*, 5 (Columbus, Ohio, 1950)
23. Bonner, J., *Plant Biochemistry* (Academic Press, New York, N. Y., 1950)
24. Bullock, R. M., *Western Fruit Grower*, **5**(4), 32 (1951)
25. Burr, G. O., and Ashton, F. M., *Repts. Hawaiian Sugar Technol., 6th Meeting*, (1948)
26. *Chemical Eng. News*, **29**, 4093 (1951)
27. Clark, B. E., and Wittwer, S. H., *Plant Physiol.*, **24**, 555 (1949)
28. Clements, J. R., and Pentzer, W. T., *Proc. Am. Soc. Hort. Sci.*, **55**, 172 (1950)
29. Coombe, B. G., *J. Australian Inst. Agr. Sci.*, **16**(2), 69 (1950)
30. Cori, C. F., *Biochem. Soc. Pub., Rept. 1st Intern. Congr. Biochem.*, 9 (1950)
31. Crafts, A. S., *Science*, **108**, 85 (1948)
32. Crane, J. C., and Blondeau, R., *Plant Physiol.*, **24**, 44 (1949)
33. Crane, J. C., and Blondeau, R., *Proc. Am. Soc. Hort. Sci.*, **54**, 102 (1949)
34. Crane, J. C., and Blondeau, R., *Plant Physiol.*, **26**, 136 (1951)
35. DeTar, J. E., Griggs, W. H., and Crane, J. C., *Proc. Am. Soc. Hort. Sci.*, **55**, 137 (1950)
36. Duhamet, L., *Compt. rend. soc. biol.*, **144**, 59 (1950)
37. Eames, A. J., *Am. J. Botany*, **37**, 840 (1950)
38. Eaton, F. M., *Botan. Gaz.*, **101**, 700 (1940)
39. Edgerton, L. J., and Hoffman, M. B., *Proc. Am. Soc. Hort. Sci.*, **57**, 120 (1951)
40. Editorial, *Agr. Chemicals*, **6**(1), 29 (1951)
41. Erickson, L. C., and Brannaman, B. L., *Proc. Am. Soc. Hort. Sci.*, **56**, 79 (1950)
42. Evans, H., (Personal communication, 1950)
43. Facey, V., *New Phytol.*, **49**, 103 (1950)
- 44a. Frey-Wyssling, A., *Submicroscopic Morphology* (Elsevier Publishing Co., Inc., New York, N. Y., 255 pp., 1948)
45. Frith, H. J., *J. Australian Inst. Agr. Sci.*, **16**(3), 101 (1950)
46. Galston, A. W., and Baker, R. S., *Am. J. Botany*, **38**, 190 (1951)
47. Galston, A. W., *Abstracts Am. Soc. Plant Physiol. Meeting*, 5 (Minneapolis, Minn., 1951)
48. Gardner, F. E., in *Plant Growth Substances*, 207 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)

49. Gates, C. M., *Proc. Florida State Hort. Soc.*, **62**, 220 (1949)
50. Gawadi, A. G., and Avery, G. S., Jr., *Am. J. Botany*, **37**, 172 (1950)
51. Goldacre, P. L., *Australian J. Sci. Research B* **2**, 154 (1949)
52. Goodwin, R. H., and Taves, C., *Am. J. Botany*, **37**, 224 (1950)
53. Gordon, S. A., and Sanchez Nieva, F., *Arch. Biochem.*, **20**, 367 (1949)
54. Gregory, L. E., *Turrialba*, **1**(4), 201 (1951)
55. Gustafson, F. G., in *Plant Growth Substances*, 351 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
56. Hall, W. C., *Plant Physiol.*, **26**, 502 (1951)
57. Hamner, C. L., Lucas, E. H., and Sell, H. M., *Mich. Agr. Expt. Sta. Quart. Bull.*, No. 29(4), 337 (1947)
58. Hamner, C. L., Sell, H. M., Klomparens, W., and Vaughn, J. R., *Botan. Gaz.*, **112**, 135 (1950)
59. Hamner, K. C. (Personal communication, 1951)
60. Hansch, C., and Muir, R. M., *Plant Physiol.*, **25**, 389 (1950)
- 60a. Hansch, C., Muir, R. M., and Metzenberg, R. L., *Plant Physiol.*, **26**, 812 (1951)
61. Harley, C. P., Marth, P. C., and Moon, H. H., *Proc. Am. Soc. Hort. Sci.*, **55**, 190 (1950)
62. Hartley, C. W. S., *World Crops*, **3**(8), 289 (1951)
63. Hartmann, H. T., *Calif. Agr.*, **3**, 4 (1949)
64. Hartmann, H. T., *Abstracts Am. Soc. Hort. Sci. Meeting*, 2 (Los Angeles, California, 1951)
65. Hempill, D. D., *Proc. Am. Soc. Hort. Sci.*, **54**, 261 (1949)
66. Hibbard, A. D., and Murneek, A. E., *Proc. Am. Soc. Hort. Sci.*, **56**, 65 (1951)
67. Hildebrandt, A. C., in *Plant Growth Substances*, 391 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
68. Hill, E. V., and Carlisle, H., *J. Ind. Hyg. and Toxicol.*, **29**(2), 85 (1947)
69. Hoehne, W., and Wasicky, R., *Rev. Quim. Farm, Rio de Janeiro*, **15**, 141 (1950)
70. Hoagland, D. R., *Inorganic Plant Nutrition* (Chronica Botanica, Waltham, Mass., 1944)
71. Holley, R. W., Boyle, F. P., and Hand, D. B., *Arch. Biochem.*, **27**, 143 (1950)
72. Hruschka, H. W., and Kaufman, J., *Proc. Am. Soc. Hort. Sci.*, **54**, 438 (1949)
73. Hsiang, T.-H. T., *Plant Physiol.*, **26**, 708 (1951)
74. Isenberg, F. M. R., Odland, M. L., Popp, H. W., and Jensen, C. O., *Science*, **113**, 58 (1951)
75. Jagendorf, A. T., and Bonner, D., *Abstracts Am. Soc. Plant Physiol. Meeting*, 8 (Columbus, Ohio, 1950)
76. Josephson, L. M., *Agronomy J.*, **43**, 404 (1951)
77. Kennedy, E. J., and Smith, O., *Am. Potato J.*, **28**, 701 (1951)
78. Kerr, T., in *Plant Growth Substances*, 37 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
- 78a. Kertesz, Z. I., *The Pectic Substances* (Interscience Publishers, Inc., New York, N. Y., 1951)
79. Knowlton, G. K., *Am. Bee J.*, **87**(9), 434 (1947)
80. Kruyt, W., and Veldstra, H., *Landbouwkund. Tijdschr.*, **63**, 398 (1951)
81. Kutsky, R. J., and Rawlins, T. E., *J. Bact.*, **60**, 763 (1950)
82. Larsen, P., *Ann. Rev. Plant Physiol.*, **2**, 169 (1951)
83. Larsen, P., *Plant Physiol.*, **26**, 697 (1951)
84. Leaper, J. M. F., and Bishop, J. R., *Botan. Gaz.*, **112**, 250 (1951)
85. Levine, M., *Botan. Gaz.*, **112**, 281 (1951)
86. Levine, M., *Am. J. Botany*, **38**, 132 (1951)
87. Lilleland, O., *Calif. Fruit and Grape Grower*, **4**(3), 23 (1950)

88. Limasset, P., Levieil, F., and Sechet, M., *Compt. Rend. Acad. Sci. Paris*, **227**, 643 (1948)
89. Luckwill, L. C., *Ann. Rept. Long Ashton Research Sta.*, 25 (1948)
90. Luckwill, L. C., *Ann. Rept. Long Ashton Research Sta.*, 124 (1948)
91. Marshall, E. R., and Smith, O., *Am. Potato J.*, **27**(4), 133 (1950)
92. Marth, P. C., and Schultz, E. S., *Am. Potato J.*, **27**(1), 23 (1950)
93. Marth, P. C., Havis, L., and Prince, V. E., *Proc. Am. Soc. Hort. Sci.*, **55**, 152 (1950)
94. Marth, P. C., Harley, C. P., and Havis, A. L., *Science*, **111**, 331 (1950)
95. McIlrath, W. J., *Botan. Gaz.*, **112**, 221 (1950)
96. McNew, G. L., and Hoffman, O. L., *Iowa State Coll. J. Sci.*, **24**, 189 (1950)
97. Meyerhof, O., *Am. Scientist*, **39**, 682 (1951)
98. Miller, I. H., and Burris, R. H., *Am. J. Botany*, **38**, 547 (1951)
99. Mitchell, J. E., Burris, R. H., and Riker, A. J., *Am. J. Botany*, **36**, 368 (1949)
100. Mitchell, J. W., in *Plant Growth Substances*, 141 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
101. Mitchell, J. W., Kraus, E. J., and Whitehead, M. R., *Botan. Gaz.*, **102**, 97 (1940)
102. Mitchell, J. W., and Marth, P. C., *Growth Regulators* (University of Chicago Press, Chicago, Ill., 129 pp., 1947)
103. Morel, G., and Wetmore, R. H., *Am. J. Botany*, **38**, 138 (1951)
104. Muir, R. M., Hansch, C. H., and Gallup, A. H., *Plant Physiol.*, **24**, 359 (1949)
105. Muir, R. M., and Hansch, C., *Plant Physiol.*, **26**, 369 (1951)
106. Murneek, A. E., *Proc. Am. Soc. Hort. Sci.*, **55**, 127 (1950)
107. Muzik, T. J., Loustalot, A. J., and Cruzado, H. J., *Agronomy J.*, **43**, 149 (1951)
108. Nance, J. F., and Cunningham, J. W., *Science*, **112**, 170 (1950)
109. Naylor, A. W., *Proc. Natl. Acad. Sci. U. S.*, **36**, 230 (1950)
110. Naylor, A. W., *Abstracts Am. Soc. Plant Physiol. Meeting*, 10 (Columbus, Ohio, 1950)
111. Naylor, A. W., and Davis, E. A., *Bull. Torrey Botan. Club*, **78**, 73 (1951)
112. Neeley, W. B., Ball, C. D., Hamner, C. L., and Sell, H. M., *Plant Physiol.*, **25**, 525 (1950); see also *Errata*, **26**(4), (1951)
113. Nelson, R. T., *Proc. Am. Soc. Sugar Beet Technol.*, **6**, 396 (1950)
114. Newcomb, E. H., *Proc. Soc. Exptl. Biol. Med.*, **76**, 504 (1951)
115. Newcomb, E. H., in *Plant Growth Substances*, 417 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
116. Newman, A. S., and Thomas, J. R., *Soil Sci. Soc. Am. Proc.*, **14**, 160 (1949)
117. Nitsch, J. P., *Am. J. Botany*, **37**, 211 (1950)
118. Nitsch, J. P., *The Role of Plant Hormones in Fruit Development* (Doctoral thesis, California Institute of Technology, Pasadena, Calif., 1950)
119. Nitsch, J. P., *Abstracts Am. Soc. Plant Physiol.*, 22 (Minneapolis, Minn., 1951)
120. Nitsch, J. P., *Am. J. Botany*, **38**, 566 (1951)
121. Norman, A. G., Minarik, C. E., and Weintraub, R. L., *Ann. Rev. Plant Physiol.*, **1**, 141 (1950)
122. Osborne, D. J., and Wain, R. L., *J. Hort. Sci. (London)*, **26**, 60 (1950)
123. Osborne, D. J., and Wain, R. L., *J. Hort. Sci. (London)*, **26**, 317 (1950)
124. Osborne, D. J., *Abstracts Am. Soc. Plant Physiol. Meeting*, 10 (Los Angeles, Calif., 1951)
125. Payne, M. G., Fuels, J. L., and Hay, R. J., *Science*, **114**, 204 (1951)
126. Porteous, H., *Calif. Farmer*, **193**(13), 523 (1950)
127. Price, C., Stewart, W. S., and Erickson, L. C., *Proc. Am. Sugar Beet Technol.*, No. 130 (1950)

128. Pucher, G. W., Leavenworth, C. S., Ginter, W. D., and Vickery, H. B., *Plant Physiol.*, **23**, 123 (1948)
129. Pucher, G. W., et al., *Plant Physiol.*, **24**, 610 (1949)
130. Reece, P. C., Furr, J. R., and Cooper, W. C., *Am. J. Botany*, **36**, 734 (1949)
131. Rhodes, A., Sexton, W. A., Spencer, L. G., and Templeman, W. G., *Research (London)*, **3**, 189 (1950)
- 131a. Rhodes, A., and Ashworth, R. de B., *Nature*, **169**, 76 (1952)
132. Rice, E. L., *Botan. Gaz.*, **112**, 207 (1950)
133. Rietsema, J., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **52**, 1039 (1949)
134. Salter, R. M., *U. S. Dept. Agr., Rept. Bur. Plant Ind.*, 58 (1950)
135. Salter, R. M., *U. S. Dept. Agr., Rept. Bur. Plant Ind.*, 73 (1950)
136. Salter, R. M., *Agr. Chemicals*, **6**(5), 48 (1951)
137. Sampson, H., *Botan. Gaz.*, **66**, 32 (1918)
138. Seeley, R. C., and Wain, R. L., *J. Hort. Sci. (London)*, **25**, 264 (1950)
139. Sideris, C. P., Young, H. Y., and Chun, H. H. Q., *Plant Physiol.*, **23**, 38 (1948)
140. Simon, E. W., *Nature*, **166**, 343 (1950)
141. Simon, E. W., and Beevers, H., *Science*, **114**, 124 (1951)
142. Shoji, K., Addicott, F. T., and Swets, W. A., *Plant Physiol.*, **26**, 189 (1951)
143. Singletary, C. C., and Warren, G. F., *Proc. Am. Soc. Hort. Sci.*, **57**, 225 (1951)
144. Skoog, F., *Ann. Rev. Biochem.*, **16**, 529 (1947)
145. Skoog, F., Editor, *Plant Growth Substances* (University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
146. Skoog, F., and Tsui, C., in *Plant Growth Substances*, 263 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
147. Sloep, A. *Onderzoekingen over pektine stoffen* (Doctoral thesis, Delft, Holland, 1928)
148. Smith, F. G., in *Plant Growth Substances*, 111 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
149. Smith, F. G., Hammer, C. L., and Carlson, R. F., *Plant Physiol.*, **22**, 58 (1947)
150. Smith, O., Ellison, J. H., and McGoldrick, F., *Science*, **109**, 66 (1949)
151. Somers, G. F., *Plant Physiol.*, **26**, 1 (1951)
152. Stahler, L. M., *Agr. Chemicals*, **6**(4), 39 (1951)
153. Steinberg, R. A., *Science*, **110**, 714 (1949)
154. Stewart, F. C., and Caplin, S. M., *Science*, **113**, 518 (1951)
155. Stewart, W. S., *Calif. Agr.*, **3**(6), 7 (1949)
156. Stewart, W. S., *Proc. Am. Soc. Hort. Sci.*, **54**, 109 (1950)
157. Stewart, W. S., and Hield, H. Z., *Proc. Am. Soc. Hort. Sci.*, **55**, 163 (1950)
158. Stewart, W. S., Klotz, L. J., and Hield, H. Z., *Calif. Agr.*, **5**(8), 4 (1951)
159. Stewart, W. S., Klotz, L. J., and Hield, H. Z., *Citrus Leaves*, **31**(8), 14 (1951)
160. Struckmeyer, B. E., in *Plant Growth Substances*, 167 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
- 160a. Syring, R. H., *The Wall Street J.*, Pacific Coast Edn., Dec. 10, 1 (1951)
161. Teubner, F. G., and Murneek, A. E., *Abstracts Am. Soc. Plant Physiol. Meeting*, 4 (Minneapolis, Minn., 1951)
162. Thimann, K. V., in *The Hormones I*, 5 (Pincus, G., and Thimann, K. V., Eds., Academic Press, New York, N. Y., 886 pp., 1948)
163. Thimann, K. V., in *Plant Growth Substances*, 21 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
164. Thimann, K. V., and Behnke, J., *Cabot Foundation (Harvard) Pub.*, **1**, 1-272 (1947)
165. Thimann, K. V., and Bonner, W. D., *Am. J. Botany*, **36**, 214 (1949)
166. Thimann, K. V., and Bonner, W. D., *Proc. Natl. Acad. Sci., U. S.*, **35**, 272 (1949)

167. Thimann, K. V., Bonner, W. D., and Christiansen, G. S., in *Plant Growth Substances*, 97 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
168. Todd, G. W., *Abstracts Am. Soc. Plant Physiol. Meeting*, 2 (Minneapolis, Minn., 1951)
169. Tukey, H. B., Editor, *Plant Hormones in Agriculture* (John Wiley & Sons, Inc., New York, N. Y., in press)
170. U. S. Tariff Comm., Repts., No. 50 (1949)
171. Veldstra, H., and Booi, H. L., *Biochim. et Biophys. Acta*, **3**, 278 (1949)
172. van Overbeek, J., *Botan. Rev.*, **5**, 660 (1939)
173. van Overbeek, J., *Ann. Rev. Biochem.*, **13**, 631 (1944)
174. van Overbeek, J., *Econom. Botany*, **1**, 446 (1947)
175. van Overbeek, J., in *Agricultural Chemistry I, Principles*, 422 (Frear, D. E. H., Ed., D. Van Nostrand Co., Inc., New York, N. Y., 812 pp., 1950)
176. van Overbeek, J., in *Plant Growth Substances*, 225 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
177. van Overbeek, J., in *Plant Hormones in Agriculture* (John Wiley & Sons, Inc., New York, N. Y., in press)
178. van Overbeek, J., Conklin, M. E., and Blakeslee, A. F., *Science*, **94**, 350 (1941)
179. van Overbeek, J., and Cruzado, H., *Plant Physiol.*, **23**, 282 (1948)
180. van Overbeek, J., Blondeau, R., and Horne, V., *Am. J. Botany*, **38**, 589 (1951)
181. van Overbeek, J., Blondeau, R., and Horne, V., *Plant Physiol.*, **26**, 687 (1951)
182. van Raalte, M. H., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **54**, 21 (1951)
183. von Denffer, D., and Gründler, H., *Biol. Zentr.*, **69**, 272 (1950)
184. Vyvyan, M. C., *Ann. Applied Biol.*, **36**, 553 (1949)
185. Wagenknecht, A. C., Riker, A. J., Allen, T. C., and Burris, R. H., *Am. J. Botany*, **38**, 550 (1951)
186. Wain, R. L., *J. Hort. Sci. (London)*, **25**, 249 (1950)
187. Wasscher, J., *Meded. Direct. Tuinbouw, Holland*, **10**, 547 (1947)
188. Weaver, R. J., and Williams, W. O., *Abstracts Am. Soc. Plant Physiol. Meeting*, 11 (Los Angeles, California, 1951)
189. Weintraub, R. L., Brown, J. W., Fields, M., and Rohan, J., *Am. J. Botany*, **37**, 682 (1950)
190. Went, F. W., *Arch. Biochem.*, **20**, 131 (1949)
191. Went, F. W., and Thimann, K. V., *Phytohormones* (Macmillan, New York, N. Y., 294 pp., 1937)
192. Wester, H. V., and Marth, P. C., *Science*, **111**, 611 (1950)
193. Wetmore, R. H., and Wardlaw, C. W., *Ann. Rev. Plant. Physiol.*, **2**, 269 (1951)
194. White, D. G., *Science*, **111**, 303 (1950)
195. Wildman, S. G., Cheo, C. C., and Bonner, J., *J. Biol. Chem.*, **180**, 985 (1949)
196. Wittwer, S. H., *Abstracts Am. Soc. Plant Physiol. Meeting*, 5 (Columbus, Ohio, 1950)
197. Wittwer, S. H., in *Plant Growth Substances*, 365 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
198. Wittwer, S. H., and Sharma, R. C., *Science*, **112**, 597 (1950)
199. Wittwer, S. H., Sharma, R. C., Weller, L. E., and Sell, H. M., *Plant Physiol.*, **25**, 539 (1950)
200. Wittwer, S. H., and Schmidt, W. A., *Proc. Am. Soc. Hort. Sci.*, **55**, 335 (1950)
201. Wittwer, S. H., and Hansen, C. M., *Agronomy J.*, **43**, 340 (1951)
202. Zimmerman, P. W., in *Plant Growth Substances*, 175 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
203. Zimmerman, P. W., *Contrib. Boyce Thompson Inst.*, **16**, 209 (1951)

TRANSPORT OF ORGANIC COMPOUNDS^{1,2}

By W. H. ARISZ

Botanical Institute, University, Groningen, Netherlands

Many reviews of the different aspects of translocation have appeared. After the well-known book of Curtis (39) in 1935, Curtis & Clark (40) in 1950 discussed the more recent literature. Mason & Phillis (67) reviewed their work done in Trinidad with cotton. Crafts (34, 37) gave two reviews, one in 1939 and the other in 1951, of the translocation of viruses, auxins, chemical indicators, and organic substances. His second article may be consulted for critical information on recent work. The articles of Huber [e.g. (54)] in the *Fortschritte der Botanik* are of great value. Anatomical data have been reviewed by Esau (44, 45). A discussion of the translocation of inorganic nutrients was given by Robertson (84, 85). Some publications with critical discussions of literature may be mentioned here (7, 22, 33, 46, 53, 88, 93, 100).

Translocation deals with the movement of substances in the cell, in parenchyma, and between different organs. Because of its large capacity this last process has attracted considerable attention. Since the tissues concerned in transport have been carefully investigated, research is now directed mostly to the transport mechanism. An osmotic theory of the mechanism, proposed by Münch (73), gave rise to much critical research. The weak points of this theory were clearly exposed by Curtis and Schumacher (100). In addition, other theories of transport by activated-diffusion and by protoplasmic streaming were put forth [Mason & Phillis (67), Schumacher (100), Curtis (39)], but the critical discussions have not given rise to any general viewpoints.

TRANSPORT IN PARENCHYMATOUS TISSUE

We will begin with transport in parenchymatous tissue in order to get a basis for a better insight into the conduction through the sieve tubes. It may be expected that transport in the sieve tubes is not fundamentally different from that in parenchyma, inasmuch as the sieve tubes are more or less differentiated parenchyma cells. In the specialised older sieve tubes other methods of transport, such as mass flow of the sap, may prevail.

Little is known about transport in parenchyma cells, especially of organic substances. A large number of substances have to be moved from cell

¹ The survey of the literature pertaining to this review was concluded in September, 1951, but it must not be regarded as a complete survey.

² It appears that inorganic substances are transported in the same fashion as organic compounds and, further, there is a possibility that they are bound to organic carriers. For these reasons the transport of inorganic substances has been discussed here insofar as it elucidates the general transport problems.

to cell, coming from or going to the channels of transport. The movement can take place in different parts of the cell, in the wall, in the protoplasm, and in the vacuole. Münch (73) assumes that transport in parenchymatous tissue occurs in the inner cytoplasm between tonoplast and plasmalemma. Plasmodesma connect the protoplasm of contiguous cells. Movement in this symplasm results from diffusion or is caused by a pressure flow which originates by an increase of osmotic substances in the cytoplasm. This brings about an increase in pressure which then forces solution through the cytoplasm into the neighbouring cells. Data supporting this hypothesis are unknown to me. In the hyphal cells of fungi, a mass flow of protoplasm takes place in the direction of the growing tip. Unilateral withdrawal of water by osmotic substances changes the direction of this flow [Buller (25)].

The removal of nitrogen compounds through different tissues from a leaf of *Pelargonium* was investigated by Schumacher (94). Transport could not be detected through the phloem parenchyma which consists of longer and broader cells than the sieve tubes. Translocation through cells of the pith over a period of 10 days was likewise small.

On the other hand, Mason & Maskell (65a) found a rather rapid translocation in the rays of the stem of cotton plants. The concentration of sugars and nitrogen compounds increased in the bark and in the adjacent xylem above a ring wound. Beneath the ring, the concentration of sugar in the xylem rapidly decreased. The rate of transport in the radial direction was seven to twelve times that of diffusion. The phloem parenchyma also reacts rapidly to concentration changes in the sieve tubes. Stout & Hoagland (106) and Chen (28), working with radioactive substances introduced through the root system, noted a rapid movement through the rays from the xylem to the bark. Tangential transport in the bark seems to be less easy (40).

Schumacher (96) found an impressive transport of foodstuffs and fluorescein through the parenchyma cells of the haustorial apparatus of the parasite *Cuscuta*. These cells penetrate between the sieve tubes of the host and take up the exuded substances through their walls, which have no plasmodesma. The cells of this apparatus are rich in protoplasm with large nuclei and bring the substances to the somewhat distant sieve tubes of the parasite (102).

Of similar function are the sugar- or salt-secreting glands, which can translocate large quantities of these substances in a polar direction. In the salt glands of *Statice*, studied by Ruhland (90) and Arisz & Heikens (8a), a remarkable quantity of sodium chloride is translocated from the leaves by processes closely connected with metabolism.

The translocation of sugars through the protoplasm is insufficiently investigated. The relation of their absorption to enzymatic reactions is well known [Street & Lowe (107), Saïd (91), Hartt (49)]. This problem cannot be dealt with here. A rapid transport of carbohydrates can be demonstrated by means of radioactive carbon (28, 81).

Vital stains as indicators of transport.—Uptake and transport of vital stains, especially of fluorochroms, have been investigated by Schumacher

(96, 97), Strugger (108, 109), and Rouschal (88). The dye is adsorbed by the cytoplasm or the wall in accordance with their electric charge; the pH, therefore, has a great influence. It is interesting that the colouring of the vacuole by acridinorange is caused by substances present in the vacuolar sap combining chemically with the penetrating molecules of the dye [Höfler (52)]. The absorption of sulfoacid stains as influenced by oxygen was studied by Collander & Holmström (29).

Fluorescein is often used in translocation experiments. It is an innoxious acid stain which is adsorbed by positively charged particles. Because of their lipid solubility, the molecules penetrate easily into the protoplasm and the vacuole. The cytoplasm absorbs the dye at pH's less than 5 but sometimes also at pH 7. The fluorescence of the wall is not brought about by adsorption, as the wall can only adsorb the stain below its isoelectric point at a pH of about 2.7. If the wall is diffusely coloured and the dye is then adsorbed by the cytoplasm, it is withdrawn from the wall. The vacuole is never intensely coloured. Since this dye penetrates so easily, one has to be cautious about inferring the path of transport from the place where the dye is adsorbed. Vital stains can hardly be of value as tracers for transport.

Schumacher (97) investigated the movement of fluorescein and aesculine in hairs of *Cucurbita* and *Primula*. They are adsorbed independently by the cytoplasm. The rate of movement is influenced by temperature and by the concentration, but it is always less than free diffusion. The distance covered is proportional to the square root of the time. This points to a diffusion process. The diffusion can be followed by the adsorption in the protoplasm; it is independent of the protoplasmic streaming. Schumacher is of the opinion that the diffusion occurs exclusively in the cytoplasm. In leaves of *Helodea*, Strugger (108) observed movement of fluorescein in the wall, probably in submicroscopical ducts. The dye rapidly passes through the walls of the midrib because of suction forces (16, 58, 108). Rouschal & Strugger (89) demonstrated the movement of potassium thiocyanate through the walls of mesophyll cells by means of a reaction with berberine sulfate. These experiments indicate that transfer in the cell wall may be effected by suction forces. The function of the wall in the transfer of solutes has also been stressed by Crafts (31). Hülsbruch (58) saw a rapid migration of berberine sulfate in the walls of root cells, independent of the movement of water. This problem needs further research.

Movement of salts and organic substances.—Bierberg in 1909 ascertained that in *Vallisneria* leaves the translocation of lithium is accelerated by protoplasmic streaming. This was not confirmed by Kok in 1931. More recent investigations indicate that accelerated movement can be obtained but without direct relation to protoplasmic streaming [Arisz & Berger (9)].

The translocation of asparagine and caffeine in leaves of *Vallisneria* was studied quantitatively by Arisz & Oudman (11, 12). Asparagine, although not penetrating by permeation into the vacuole, is moved more rapidly and over a greater distance than caffeine which enters easily. The movement of as-

paragine is only slightly enhanced by a suction brought about by increasing the transpiration of a part of the leaf. The uptake of asparagine is an accumulation process resulting in a higher osmotic value of the cell sap [Arisz & van Dijk (13)]. This is not the sequence of osmoregulation as absorbed asparagine can be given off by exosmosis. Passive permeation of asparagine cannot be demonstrated with the deplasmolysis methods of Höfler (52a) and of Collander & Bärlund (28a). Consequently, the movement into the vacuole has to be an active process. Withholding oxygen inhibits further uptake, although it does not bring about exosmosis of the accumulated asparagine. Exosmosis can be obtained by transferring leaves from a higher to a lower concentration, especially when the leaves have little resistance. Temporarily, the protoplasm may become permeable, and asparagine diffuses outward or inward, depending on the concentration gradient [Arisz (5)]. After a short time the original semipermeability is restored.

Vallisneria leaves likewise absorb and transport chloride [Arisz (8)]. The salt uptake is the same as that of asparagine, except in one respect—the sensitivity to light. The translocation of chloride can be investigated by local absorption by a part of the leaf and transport to the rest. Wounding inhibits this process, but after a pretreatment in streaming water for 24 hr. this inhibition disappears. When the absorbing zone remains in the dark and the free part of the leaf is placed in the light, the latter part absorbs more chloride than the contact zone. This ensues from the passage of chloride to the free part of the leaf and its accumulation there in the light. The contact zone is in the dark, and can only slightly accumulate it. From this, it follows that the salt is moved outside the vacuole and that this process is extremely sensitive to a wound stimulus. This points to transport in the cytoplasm. Some time ago, Rouschal (88) suggested that such a transport could occur in the cell wall at the boundary of the cytoplasm and could be activated there by the cytoplasm. For the present, the assumption that the transport is in the cytoplasm and that the substances are secreted into the vacuole seems the most appropriate.

When a part of the leaf has absorbed chloride in the vacuoles and is then placed in water, there is no further distribution of the absorbed chloride to the vacuoles in the rest of the leaf (8). This indicates that there is no direct communication by intervening cytoplasm between the vacuoles of contiguous cells. Transport in the cytoplasm and secretion into the vacuole are separate processes.

Drosera.—Tentacles of *Drosera* are organs which absorb different substances with their glands and translocate them by means of their pedicels to the leaf [Oudman (76)]. Assuming that transport in parenchymatic tissue can be studied most efficiently in specialized organs, Arisz and his co-workers have extensively investigated this process. It could be demonstrated that the transport of caffeine behaves like a diffusion process [Kok (59a)]. It can be followed easily by the formation of coacervate drops in the vacuole. The diffusion proceeds in longitudinal cell rows from cell to cell. Surely this

transport does not follow the side walls, as the neighbouring cells need not show any precipitation. While caffeine moves through the vacuole, asparagine is translocated in the cytoplasm. This is indicated by the aggregation of the cytoplasm and the dehydration of the vacuole (10).

It can be shown that this transport is independent of a simultaneous water transport (4). An increase in the transpiration of the leaf does not influence the asparagine transport. Likewise, the addition of sugar or salt in high concentration to the agar from which the substances have to be absorbed by the glands is without influence, although the water now moves in the opposite direction to that of asparagine. When the concentration is sufficiently high, the tentacles lose their turgor and the power of curvation. They keep their position between the agar strips and are thus well suited for translocation experiments. If the osmotic concentration is too high, the transport is inhibited.

The following substances can be moved: asparagine and various amino acids, phosphate, and ammonium carbonate. Organic substances like urea, thiomethylurea, phenylurea, various amides, caffeine, and antipyrine are also moved. Sugars, chloride, and nitrate are not transported. This translocation is more or less sensitive to withdrawal of oxygen with regard to the above-mentioned substances. This proves that it is never a pure diffusion process, even when the rules of diffusion are followed. Therefore, it is called activated transport by Arisz (4). The degree of activation depends on the nature of the substance and is probably greater for substances that cannot readily diffuse through the cytoplasm, e.g., phosphate, amino acids, and asparagine. A correlation seems to exist between the power of a substance to cause aggregation and the activity of the transport.

The quantity of substance which is present in the tentacles themselves can be neglected with regard to the quantities moved to the leaves. Computed on the water present in the leaves, the amount of transported substance gives a concentration much larger than that of the substance in the medium. This accumulation factor for transport of glycol from a 0.2 mM solution amounts to 135 after 42 hr.

The selectivity of the uptake indicates affinity of the cytoplasm for the absorbed substance. Hence, it was accepted that actively transported substances are bound to cytoplasmatic carriers [Arisz (6)].

From experiments on simultaneous transport of different substances some general rules could be formulated (6). The cytoplasm does not distinguish between different amino acids nor between these and asparagine. Simultaneous transport of an amino acid and phosphate, on the contrary, gives rise to summation. This points to independent transport processes for phosphate and amino acids.

Influence of inhibitors.—Continuing these researches, Arisz (3a) investigated the influence of different substances, known as inhibitors of enzymatic processes, on the transport of phosphate and of asparagine. Some of the results may be mentioned here. Potassium cyanide, azide, iodoacetate, fluo-

ride, arsenite, and 2,4-dinitrophenol inhibit the transport of both substances. There is one exception. Phloridzine inhibits the transfer of phosphate only (10^{-3} mole gives 50 per cent inhibition). This indicates that phosphate is bound by an enzyme system. It reminds one of an oxidative decarboxylation of pyruvic acid coupled with a phosphorylation of adenylic acid, as studied by Shapiro (103). The other inhibitors influence a general process common to the translocation of both substances.

Tentacles without glands do not transport asparagine, at least when in low concentration. They do transport phosphate. This transport is neither inhibited by phloridzine nor by iodoacetate, fluoride, and dinitrophenol. The inhibition by these substances in tentacles with glands must, therefore, be that of enzymatic processes in the gland. Yet the movement of phosphate in the pedicles is not a pure diffusion process since it is inhibited by oxygen withdrawal. In complete agreement with this it could be shown that cyanide and azide inhibit this transport. Since these substances inhibit the cytochrome oxidase system, this means that the energy for the active transport is provided by this part of the respiration process. Since 2,4-dinitrophenol does not inhibit the movement through the tentacles without glands, the energy-providing phosphorylation found by Robertson (85) is not involved.

We have observed in parenchymatous tissue different methods of transport ranging from true diffusion to more or less activated transport dependent on metabolism. An important result is the fundamental similarity in the transport of phosphate and some polar organic substances like amino acids, asparagine, and others.

The problem is particularly interesting if the substances are translocated combined with carriers. Evidently enzymatic processes are involved in the uptake and the movement. It may be that the transport proper in the cytoplasm is directly connected with the cytochrome oxidase system. Robertson (85) mentioned a transport of ions in mitochondria and microsomes.

Translocation is not polar in *Vallisneria* leaves but it is in the roots, in *Drosera* tentacles, and in salt glands. The cause may be located either in the tissue or in active mechanisms outside [Went (119)].

TRANSPORT IN THE ROOT

Uptake and transport of anions in the root depend on the anion respiration and the cytochrome oxidase system [Lundegårdh (63), Robertson (84) Weeks & Robertson (117)]. The above-mentioned data on *Drosera* support this conception as far as transport is concerned.

Recent experiments of Sandström (92) with di-*n*-amylacetic acid have shown that this substance causes the epidermal cells of the root to peel off without affecting the cortical parenchyma. Thus, this makes it possible to determine whether or not salts have to be absorbed by the epidermis before they can be moved to the xylem. In roots without epidermis, the ions moved passively with the water and passed into the xylem vessels in the same concentration as in the external solution. Selective absorption seems to have

disappeared. The cells surrounding the xylem pump the solution into the vessels without any selective action. This problem needs further research since it is known that salts and stains can be moved in the walls of epidermal and cortical cells.

Lundegårdh (63) mentions experiments in which neutral red is absorbed by roots. His assumption that the vital staining marks the pathway of mineral solutes cannot be accepted. The dye was absorbed by the epidermis, passed through the cortex, and went in a radial direction to the stele. The vacuoles in the elongation region were stained and the dye was fixed in the lignified walls of the vessels. It is known that neutral red molecules easily penetrate into the cytoplasm and the vacuole. The driving force of this process is a diffusion gradient sustained by the adsorption of the dye in the wall of the xylem vessels. This can give no indication of the transport of salts as these are moved by active processes through the cytoplasm and are actively secreted into the vacuole or into the xylem.

Lundegårdh is of the opinion that anion respiration is needed for the entrance of anions into the cytoplasm and into the vacuole and that the ions on their way to the xylem have to pass through the vacuoles. For this reason experiments indicating that the pathway of ions is outside the vacuoles are of importance [Arisz (7, 8)]. The data of Broyer (24) concern the movement of radioactive bromide ions into the vacuoles and into the xylem. Though the exchange between radioactive ions in the cytoplasm and in the medium is easy, ions once secreted into the vacuole do not exchange readily with those passing in the cytoplasm on their way to the xylem. This indicates impermeability of the tonoplast and an active secretion of these ions into the vacuole. Broyer assumes that the salt is moved to the xylem in the cytoplasm from cell to cell without entering the vacuoles. A secretion into the vacuoles takes place according to the quantity of ions already present.

The root cells can give off ions and other substances to the medium. This happens especially when the plant is in a phase of declining vegetative growth in which salts are released from decaying cells and transported to the root [Gregory (48)]. There is a release of potassium by the root if the plant remains in the dark for more than 12 hr. [Luttkus & Böttcher (64), van Andel, Arisz & Helder (2)]. This phenomenon is important as it shows that the root can release salts as well as absorb them.

Wiersum (121) studied the movement of substances across the root of *Vicia faba* from the medium to the xylem and in the reverse direction. Salts go as well to the medium as into the root under the influence of a concentration gradient or a suction stream. He also found transport of sucrose and urea. His results point to a rather easy diffusion of substances in both directions in the root of this plant. The researches of Arisz *et al.* (9a, 75) confirmed the opinion of Lundegårdh (62) that the transport of salt ions to the xylem is not based on permeability but is a process dependent on metabolism. Lundegårdh is of the opinion that the active cells are situated contiguous to xylem vessels. This has not been proved yet. In any case, salt secre-

tion into the xylem is an active process independent of the secretion into the vacuoles of the cortical cells. Other substances than salt may also be transferred by the transpiration stream, e.g., nicotine [Dawson (42)].

SALT TRANSPORT IN THE PLANT

Tracers.—Since the uptake of salts is easily demonstrated and the transport of such ions as rubidium and bromine, which are absent in normal plants, can be studied, there is better information about the transport of salts than of amino acids, carbohydrates, etc. The use of radioactive isotopes has opened new prospects [Burris (26)]. They are valuable for studying ion exchange. At low temperatures where active processes are largely ruled out, the strength of ion exchange can be assessed. Hence, when working with radioactive substances in transport experiments, there is the inherent drawback that at higher temperatures active transport and ion exchange can not be separated and the impression of transport can be created where ion exchange takes place [cf. Steinbach (104)]. There is a wide field for the application of tracers in view of the intimate connection of transport with metabolic processes.

Circulation of salts.—There is agreement that salts circulate in the plant [Mason, Maskell & Phillis (66), Biddulph (18), Biddulph & Markle (20), Crafts (33), Moore (72)]. The transpiration stream brings salts secreted into the xylem of the root to all transpiring parts of the plant, while the cells adjoining the xylem vessels take up salts, keep them, or conduct them in the rays to the bark. Those salts that are not used in growth nor accumulated in the tissues are carried off to the roots by the sieve tubes. Biddulph (19) found a daily periodicity in the transport of phosphorus out of the leaf [cf. Hartt (49)]. A surplus may be released by the roots to the medium. Radioactive phosphorus administered as potassium phosphate makes a complete cycle from root to root in 3 to 6 hr. [(29b); Moore (72)]. Apart from this circulation, there is a phosphate transport to places of utilization. Curtis (39, 40) showed that salts are transported through the phloem to growing parts. Burström (27) ascertained a transport in the phloem to young buds of *Carpinus* during the exudation period. Arnon *et al.* (14) reported transport from leaves to fruits.

Absence of calcium in sieve tubes.—Some ions, such as calcium and probably lithium (59, 67), are fixed in cells and are not transported or are transported only in small amounts by the sieve tubes to places of utilization. Reed & Haas (82) mention that *Citrus*, when deficient in calcium, cannot use the calcium present in other parts of the plant. In experiments of McAlister & Krober (68), calcium failed to move readily from the cotyledons as a result of being present in a relatively insoluble form in the cells. An observation of Curtis & Clark (40) is in conflict with this view. They suppose that calcium is conducted in the phloem to growing shoots. Their conclusion is wrong. Defoliated shoot tips of *Sumach* cannot transpire so that even their control plants could not translocate calcium. The relative increase in calcium

amounts to one-tenth of that of the phloem mobile potassium. When the phloem or the xylem is cut, there is no significant change in the quantity of calcium present; but after the xylem is cut, the tip still receives potassium. This is an argument in favour of a transport of potassium through the phloem, but not of calcium. We shall revert to this characteristic feature, i.e., that sieve tubes generally do not transport calcium.

TRANSPORT IN THE PHLOEM

Most authors seem to be of the opinion that only one principle can be valid for the transport through the sieve tubes and that one has to make a choice between the existing theories. We have learned from the discussion of transport in parenchyma cells that there are different methods by which substances are moved in the cells. The relation of this transport to metabolism and the specific inhibitions of it by some enzyme poisons made it apparent that every substance moves in its own way and that one can expect the same mechanism only for specific substances. Therefore it is *a priori* likely that in the sieve tubes, too, different transport mechanisms are involved depending on the kind of substances moved and on the degree of differentiation of the ducts.

Translocation of substances can be studied by different methods, either by quantitative determination of the transported substances or by indicators. For this purpose, vital stains can be used or radioactive elements and chemical compounds which can be estimated easily. The transport of sugar supplied to leaves has been detected by its influence on growth and exudation [Went & Carter (120)]. Transport of growth substances, e.g., 2,4-dichlorophenoxyacetic acid, is apparent from the curving of the stem.

Anatomy of the sieve tubes.—We shall begin with a discussion of the anatomy of the transport channels. Though it is incorrect to infer the function of a cell or an organ from its structure, a knowledge of the anatomical structure of the transport ducts is indispensable for a correct interpretation of the physiological data. The gymnosperms have as conducting elements sieve cells; those of the conifers, being remarkably poor in cytoplasm, normally can be plasmolyzed. Most angiosperms have sieve tubes, differing in length and diameter. The shorter the cells, the greater the number of cross walls and the resistance to the movement of substances. The elements of the vascular bundles of some immersed aquatic plants have the structure of parenchyma cells.

The structure of the transport system of some Dioscoraceae is remarkable. Mason (65) discovered that bast glomeruli are situated in the nodes which separate the sieve tubes in the successive internodes. These glomeruli contain regularly arranged parenchyma cells with much cytoplasm and give the impression of secretion cells [Brouwer (23a), Happ cited by Huber (54)]. It is apparent that this tissue has a special function which may be that of secreting substances into the sieve tubes. Schumacher (96) compares it to the haustorial apparatus of the parasite *Cuscuta*.

The sieve tubes live from several days to four years. When mature, they display profound changes which were studied extensively by Esau (45) and Crafts (37). They lose their nucleus or only parts of it remain. The cytoplasm becomes strongly hydrated and lies in a thin layer against the wall. Streaming of the cytoplasm cannot be seen in mature sieve tubes. In different objects, e.g., *Pinus strobus*, the boundary with the vacuole is invisible [Rouschal (88)]. In others, a tonoplast is present. In *Aesculus* and *Cucurbita* it is uncertain if there is a distinct vacuole. By inducing streaming of the contents, plastids and starch granules are moved. This is a point of similarity between latex vessels and sieve tubes. While young sieve tubes absorb neutral red, older ones lose their stainability (37, 56, 88).

At first, it was dubious if older sieve tubes could be plasmolyzed, but afterwards it was stated by Schumacher (99), Rouschal (88), and Curtis & Asai (39a) that they could. Crafts (35, 37) assumes that sieve tubes only function in a phase when they can no longer be plasmolyzed. From a physiological point of view, this hypothesis is not very likely since even an ordinary parenchyma cell is able to attain actively accelerated transport. More acceptable is his assumption that sieve tubes, without a tonoplast and with a cytoplasm at the sieve plates which is easily permeable, are adapted to a mass flow of the whole contents.

Much attention has been paid to the sieve plates and the pores since the resistance of these pores filled with cytoplasm was estimated to be so large that a mass flow was impossible. Münch (73) also considered the open communication through the pores of great importance for his theory, but later he reconsidered the point and was of the opinion that even if the pores are not open a mass flow is possible (74). Huber & Kolbe (55), using an electron microscope, observed a great number of sieve fields each with pores of 1 to 2μ separated by a fine meshwork situated in the sieve membrane. Sometimes the pores are extremely large, e.g., those of *Fraxinus excelsior* are 15μ [Huber (54)].

Phloem exudation.—The phenomenon of exudation of phloem sap from the sieve tubes by cutting or pricking has provoked extensive research. The flow of sap as a result of cutting was considered as a proof of a high pressure in the sieve tubes and of the opportunity for rapid transport of a solution through the sieve tubes.

From the analysis of the flow of latex from cut bark of *Hevea* trees, it is apparent that by releasing the pressure the suction tension in the vessels is increased above that of the surrounding tissue so that water is absorbed and the flow is enhanced [Arisz (3)]. One has to keep this phenomenon of osmotic suction of water in mind when systems of connected elements are opened [Frey-Wyssling (47)].

Apparently there has to be a special mechanism in the sieve tubes to prevent a continuous flow of sap at wounds. The latex vessels compose a system of connected channels with perforated septa where streaming can easily be realized, while the sieve tubes are separated by cross walls with narrow pores

filled with cytoplasm. The slime plug formation in opened sieve tubes gives the impression that by the sudden change in pressure a part of the cell contents is pressed against the sieve plate so that a barrier is formed [Crafts (33)].

Münch (73) reports that the pressure change can be observed over large distances. It is transmitted downwards in the sieve tubes of *Quercus rubra* at a rate of 10 to 20 cm. per sec. Upwards, the pressure change extends over a distance of 1 m.; downwards, over 5 to 6 m. The pressure is rapidly restored.

By repeatedly cutting, Crafts (32) obtained a flow during a 24-hr. period. The dilution reaction is apparent from the data of Crafts and of Crafts & Lorenz (38). They found by continued cutting of stems of pumpkin that the carbon content expressed as a percentage of the dry weight decreases in 4 hr. from 59.03 to 32.77 per cent, and the nitrogen content from 16.22 to 11.39 per cent. Cooil (30) found a direct relation between the water content of the tissue and the exudate. He collected the exudate of *C. pepo* during the first minute after cutting and found it diluted with water from the adjoining tissue.

Tingley (115), working with the Barger method for the determination of osmotic values, obtained unreliable values. Determinations of the sugar content with a refractometer were more accurate, but the sugar concentration depended on the sequence in which the cuts at different heights were made. She found the dilution effect to be over 2 ft. in both directions.

Significance of sap concentration.—There is a second difficulty in the appreciation of the significance of concentration determinations of the sap. It arises as the result of our ignorance of how the substances are moved in the sieve tubes, whether separately or as a solution, and whether, if it is a solution that is translocated, this takes place in the cytoplasm, in the vacuole, or in both. An impression can be obtained of the transport to developing fruits by analyzing their composition at different stages. This gives the ratio in which carbon and nitrogen are used for their growth and therefore have to be supplied. If these substances are moved in the sieve tubes, one can expect the same relation in the exuded sap. Colwell (29a) found no agreement between the carbon/nitrogen ratio in fruits and the exudate of *Cucurbita*. Crafts & Lorenz (38) report that 80 per cent of the dry weight of the sap consists of proteins. Three times more sugar is needed than nitrogen compounds for growth of the fruit. This difference is so large that it seems doubtful whether the exudation is a manifestation of normal food movement. Crafts (37) is of the opinion that trees give better concordance between the exuded and the translocated sap.

The experiments of Huber, Schmidt & Jahnel (57) with sap of *Q. rubra* are most interesting. The sugar content of the sap obtained at a height of 1.5 to 12.5 m. was determined with a refractometer. In the exuded sap they found a minimum sugar concentration at a height of from 10 to 12.5 m. at 1:00 P.M., followed by an increase. At a height of 2 m., the minimum concentration is found 4 hr. later followed also by an increase. Their data give

the impression that a concentration increase arising from the assimilating crown moves downwards at a rate of 2 m. per hr. The concentration changes in leaves as a result of photosynthesis are reduced in the trunk by the giving off of assimilates at places of storage and utilization.

It is remarkable that Leonhardt (60), by putting several aphids on a strongly assimilating branch and catching the secreted liquid of the lice on a rotating disk, obtained the same periodicity in the number of drops secreted by the lice during day and night. No rhythm was found when the leaves remained in the dark. The experiments should be repeated as Michel (69) could not corroborate the rhythmic secretion.

Mechanism of the transport in the sieve tubes.—Opinion differs on the mechanism of translocation. Mason and co-workers (67) concluded that carbohydrates and nitrogen compounds can be transported in the sieve tubes in either direction. They found concentration differences of some substances and demonstrated that when the direction of the transport was reversed the concentration differences at the same time were changed. They consider these concentration differences as the cause of the translocation and suggest an activated diffusion in the cytoplasm. It must be remarked that it seems impossible to decide if concentration differences are cause or effect of transport to and from the sieve tubes or through them.

Schumacher (95, 98) attaches great significance to the behaviour of fluorescein in the sieve tubes. He observed an accelerated diffusion dependent on a concentration gradient and calls it a molecular movement. This transport is insensitive to narcotics.

Münch (73) considers the transport in the sieve tubes to be the result of turgor differences existing between the parenchyma of the distributing and the receiving tissues. In the distributing tissue, e.g., leaves that photosynthesize, a high pressure exists in the cytoplasm, which conveys the assimilates and other substances from these cells in the sieve tubes. The substances are used or fixed in the receiving tissue so that the osmotic pressure in the cytoplasm remains low. The transport in the sieve tubes is a mass flow through the lumen caused by turgor differences. Later Münch (74) attached less importance to his hypothesis about the origin of the pressure flow in the parenchyma. He spoke of a pressure stream originating as a sequence of differences in osmotic value in different places of the plant. Frey-Wyssling (47) indicated that a flow can as well be the result of a difference of suction forces (diffusion pressure deficit).

We want to speak here of mass flow in the sieve tubes independently of the theory postulated by Münch only to indicate that a solution is moved in the sieve tubes. With this restriction we can say that Huber and Crafts have accepted the principle of mass flow. Curtis (39) and Curtis & Clark (40) have given a critical survey of the different theories. They stress the importance of plasmatic transport, and call it protoplasmic streaming but include in this term all mass movement in the cytoplasm even when there is no relation to visible protoplasmic streaming. The space available for this review only allows one to deal here with some general problems.

An important part of the discussion concerns the question of whether the differences in turgor accepted by Münch as the cause of the transport can actually be demonstrated. Differences in turgor are based on differences in osmotic value. Therefore, osmotic values at the site of distribution and of utilization have been studied by Pfeiffer (79, 80) and by Curtis & Scofield (41). Their results are contradictory. Since we know at present that the transport in parenchymatous tissue is connected with metabolism and takes place in the cytoplasm, this problem will have to be investigated again. It is still doubtful whether the osmotic value of the vacuole has anything to do with transport and whether a pressure flow in parenchyma exists.

Simultaneous transport in opposite directions.—A second problem to be discussed here is the question of whether a mass flow can be excluded by demonstrating that different substances can be simultaneously conducted in the sieve tubes in opposite directions. Much more knowledge about the mechanism of the sieve tubes has to be gathered before we can draw such a conclusion. Mason, Maskell & Phillis (66) and Mason & Phillis (67) report a simultaneous transport of carbohydrates and nitrogen compounds in opposite directions. Fischer (46), working on the leaves of *Cineraria* and *Pelargonium*, found that in the dark, protein breakdown occurs as the result of carbohydrate lack. If the lower leaves are left in the dark for some time and the rest of the plant is put in the light then the leaves in the dark lose nitrogen and take up carbohydrates. Chen (28) using radioactive C^{14} and P^{32} is also convinced that he has demonstrated simultaneous transport of carbohydrates and phosphates in opposite direction.

Such data can never prove that mass flow is impossible, since, even when it could be ascertained that the transport was simultaneous in the same sieve tubes in opposite direction, this would only prove that one of the substances moved was not transported by mass flow, but never that none of them was transported by mass flow. Proof of the simultaneousness of the transport in both directions is not always convincing. The experiments of Mason *et al.* took 14 days. The duration of the experiments of Chen was much shorter, only some hours, but it seems possible that in his experiments P^{32} goes upward in the xylem and that at places, where the stripped bark is in contact with the xylem, the tracer passes into the sieve tubes and is afterwards conducted downwards together with the carbohydrates. The investigation of Palmquist (77) on simultaneous transport of carbohydrates and fluorescein furnishes no proof against mass flow since fluorescein can certainly be translocated in another way, i.e., by diffusion.

MODELS OF MASS FLOW IN SIEVE TUBES

We have to mention data which point to the presence of mass flow in sieve tubes. A mass flow of cytoplasm in hyphal cells of fungi is known, but for parenchymatous tissue there are no data.

Bleeding of palm trees.—Firstly, the phenomenon of local "bleeding" in palm trees demands our attention. Tammes (111) reported that in male inflorescences of *Arenga* and *Cocos* the cause of the bleeding is situated in a

more or less small area stimulated by bruising some time before. Cut branches of an inflorescence of *Cocos* placed in water continue to exude slightly. The vessels in the stimulated zone are clogged and air cannot pass them. Acid fuchsin, which easily passes the xylem vessels, does not enter the bleeding sap. A solution of potassium ferrocyanide does, and can be demonstrated in the sieve tubes by using ferric chloride. It is likely that, as a consequence of the preceding stimulation, sap goes from the sieve tubes into the xylem vessels just above the place where they are clogged.

Tammes (113) reported the absence of calcium in the juice. This agrees with the experience that sieve tubes do not translocate calcium and supports the assumption that the bleeding sap is coming from the sieve tubes.

In *Arenga*, an exudation containing about 14 per cent sucrose was obtained over a period of 50 days. During that time 28 kg. of sucrose in 220 l. of sap were transported, i.e., 23 gm. sucrose and 183 cc. sap per hr. Tammes (114) calculates the total cross area of the sieve tubes as 0.34 sq. cm. This gives a rate of 7 m. per hr. Although this is an abnormal process for the plant it can be continued over a period of some months. The large quantities of stored starch in the stem are then used up. If an inflorescence is cut off before the tissue has been stimulated, or if, after stimulation, the cut is made beneath the stimulated zone, there is no exudation at all, indicating that the flow is the result of the special conditions in the small stimulated zone and that the phloem from the stem to the stimulated zone is in normal condition. The abnormality is only in the stimulated zone. The flow in the sieve tubes is continued by the mobilisation of starch in the tissues of the stem and the transformation of the starch into sucrose. This process cannot be the direct result of the local stimulation of the inflorescence but must be the consequence of the pressure diminution in the sieve tubes. This exudation seems to be a model of an induced pressure stream in the phloem. The decrease of the pressure in the sieve tubes may have caused a dilution of the sap so that the real concentration of sucrose in the sieve tubes is somewhat higher.

Mass flow in Arachis.—While this "bleeding" represents a mass flow caused by artificial conditions, there are indications that mass flow also occurs in the sieve tubes of the intact plant. Several investigators found that good fruiting of *Arachis hypogaea* could only be obtained in a soil rich in calcium and that the calcium had to be absorbed by the gynophores and the developing fruits [Bledsoe & Harris (21)]. Wiersum (122) introduced fast green, eosine, and neutral red in some lateral roots and showed that the xylem of the fruits remained colourless. This proves that transpiration cannot transfer salts to the growing fruits as long as these are enclosed in the soil and are unable to lose water by transpiration. Since there is no transpiration, salts can only be transferred to the fruits by the phloem. But, as we have seen, calcium is not translocated by the sieve tubes, therefore it has to be absorbed by the fruits themselves from the soil. Wiersum reports that the pods absorb potassium ferrocyanide from a solution. It can be detected with ferric chloride not only in the fruits but also in the xylem of the stem in two

internodes above the insertion of the fruits. This suggests a water transport from the fruit to the shoot, and it seems likely that this water has been supplied by the phloem, though this cannot be proved, since it may also come from the soil. Therefore, under normal conditions we have a flow of a solution containing salts, carbohydrates, and nitrogen compounds to the fruits, which can only take place through the sieve tubes, while the water of the solution is probably released in the xylem.

Simultaneous transport in the same direction.—While a simultaneous transport in opposite directions has been used as a proof against mass flow in the sieve tubes, we have some data regarding simultaneous transport in the same direction which may agree with it. Most interesting are data on the transport of 2,4-dichlorophenoxyacetate (2,4-D) from the primary leaves of *Phaseolus vulgaris*. This substance, introduced into the midrib of a primary leaf, is transported downwards in the sieve vessels only when there is a simultaneous transport of carbohydrates. In the dark or in the light without carbon dioxide, 2,4-D is not transferred downwards. Transport can also be obtained when the leaves absorb sugar in the dark [Weintraub & Brown (118)]. It is of no significance that glucose and maltose have the same influence since there is an interconversion of sugars in the leaf. If one of the leaves of a pair receives sugar and the other 2,4-D, no reaction results, indicating that the lack of reaction was the result of the 2,4-D not being transported, and not as the result of the lack of sugar essential for the execution of the reaction. These results indicate a mass movement caused by the transport of the sugar [Mitchell (70), Mitchell & Brown (71), Dhillon & Lucas (43), Rice (83) Rohrbaugh & Rice (87)]. Related compounds also behave in this way [Linder, Brown & Mitchell (61)].

Further data indicate that virus molecules are also transported together with carbohydrates [Bennett (17), Crafts (37), Hildebrand & Curtis (51)]. A transport of a flower-inducing hormone in the same direction as sugar was mentioned by Stout (105).

It is not valid to conclude from these data that the transport of these substances is based on mass streaming. It may also be interpreted as molecular mass transport in the cytoplasm. As it may be expected that such molecular movement will take place in a way specific for each substance, this assumption does not seem very likely. The presence of mass flow being proved, it seems acceptable that at least in the case of 2,4-D we may interpret it as such.

Flow in the sieve tubes by suction.—Some observations indicate that streaming in the sieve tubes can be obtained by suction. Rouschal (88) reported that a hypertonic glycerol solution unilaterally applied to a section of the phloem of *Aesculus* caused dislocation of the contents of the sieve tubes and movement of starch granules. He also introduced glycerol into the stem and observed a movement of fluorescein in the sieve tubes. We will revert to this point.

Both (22) tried to obtain a flow in the sieve tubes with a suction caused by transpiration. In this way solutes could be transported through the sieve

tubes to the transpiring tissue. He used excised stems of *Impatiens Marianae* with two leaves and closed the vessels of the wood over a great distance with cocoa butter. The leaves were held at different humidities and the transport of asparagine and caffeine from leaf to leaf was investigated. He found an increase of nitrogen in the transpiring leaf and gives as proof that the transport really took place through the sieve tubes, the inhibition of the transport by eosine, a substance used by Schumacher to stop transport in sieve tubes. In view of the experiments of Rouschal & Strugger (89) a transport in cell walls has also to be considered, but because of the long distance over which the vessels were filled with cocoa butter this appears to be unlikely.

Tammes (112), putting leaves of *Cocos* palms in a solution of potassium ferrocyanide during dry weather, obtained an ascent of this substance in the sieve tubes. The xylem vessels were found to be clogged by air bubbles. This also indicates a displacement of a solution in the sieve tubes and the possibility that other substances are transported simultaneously. These experiments were continued with leaves of *Ammophila* and a similar though smaller effect was obtained [Tammes (114)].

Analysis of mass flow.—The presence of mass flow has been established and it now has to be considered how mass flow in the sieve tubes can be understood. Münch accepts a displacement of the whole contents and apparently this is also the opinion of Crafts and of Huber. It seems to me that a second possibility has also to be considered, i.e., a flow through the cytoplasm outside the vacuole, as was suggested by Münch for parenchymatous tissue. A similar flow of foodstuffs has not yet been found in parenchymatous tissue, but the circumstances in the sieve tubes are much more favourable because of the better cytoplasmic communication of the sieve tubes through the sieve plates. When the sieve tubes are mature and the tonoplast begins to degenerate, the flow in the cytoplasm will gradually change to a streaming of the whole contents. This point of view has several advantages. It can account for the lack of agreement between the composition of the phloem sap and the substances needed for the growth of fruits, and, on the other hand, for the agreement between the concentration changes of the sap of trees and the periodic formation of assimilates. In the first case, there may have been streaming in the cytoplasm; in the second, a flow of the whole contents. The evolution of the ducts from parenchyma cells to specialised sieve tubes is to a certain degree repeated by every sieve tube during its differentiation. Mass flow will mostly be a temporary streaming as a consequence of the periodic entrance and removal of substances from the transport channels.

Furthermore, it has to be considered whether in addition to mass flow other transport mechanisms are also possible in the sieve tubes. As long as these elements are cells comparable to ordinary parenchyma cells, it has to be taken for granted that they possess the same faculties for transport as parenchyma cells, i.e., diffusion, activated diffusion, and transport combined with metabolism.

Transport of fluorescein in sieve tubes.—We have already discussed the

transport of fluorescein in parenchyma. Next we have to consider its transport in the sieve tubes. The translocation of fluorescein can be quite independent of that of sugar and nitrogen compounds (78, 95, 98). In cut leaves the dye spreads until close to the cut edge of the petiole. An exudation of water does not occur even when back suction by the tissue is prevented by clogging the xylem vessels with cocoa butter. This demonstrates the movement of fluorescein without a simultaneous water transport [Schumacher (101)]. The dye can be seen in favourable objects in the vacuole (88). With greater concentration, the cytoplasm cannot absorb all the dye and the lumen then gives a remarkable fluorescence [Bauer (16)]. This points to transport in both vacuole and cytoplasm. The well-known experiment of Rouschal (88) in which reversal of the direction of fluorescein transport over distances of 35 cm. by injection of concentrated glycerol into the stem or fruitstalk of *Cucurbitaceae*, indicates that the fluorescein in the sieve vessels can be displaced by osmotic suction; but it is no proof of a mass flow. This process is complicated, since sugar does not have the same effect (101). Sometimes fluorescein is moved to the points of utilization of foodstuffs with a rate equal to that of the transport of carbohydrates (88, 98). This points to mass flow. Fluorescein would then be a substance which can be moved by (activated) diffusion as well as by mass flow. The peculiarity of fluorescein is the adsorption by the cytoplasm which actually makes the substance unsuited for transport.

It seems likely that many other substances can also be translocated by diffusion (eventually activated diffusion) as well as by mass transport. The values of Chen (28) on the transport of radioactive substances are sometimes constant over a certain distance, while at other times there is a strong concentration gradient. This may indicate that sometimes mass flow prevails but, if it is absent, substances are moved by diffusion. These questions call for further investigation.

Uptake in and withdrawal of substances from the sieve tubes.—The experiments with parasites by Schumacher (96) indicate that substances are released from the sieve tubes when the haustoria penetrate between them. The haustorial cells of the parasite, rich in cytoplasm, absorb and transport these substances. It is likely that the cytoplasm of the sieve tubes of the host loses the substances at the exposed sieve fields. In the same way, sieve tubes in the normal condition may give off substances to the neighbouring parenchyma cells. They are then transported in the cytoplasm of the parenchyma cells or secreted into the vacuoles or consumed by synthesis or dissimilation processes. The downward transport from fading leaves and flowers [Schumacher (94a)] and the sudden reversal of transport in the cotton plant when the flowers begin to fade [Mason (67)] are indications that the metabolism of the organ has a great influence on transport.

The movement of substances in the leaves from the assimilating cells to the sieve tubes is a complicated problem. There is an interconversion of sugars in the leaf and according to Mason & Maskell (65a) concentration gradients causing diffusion can arise for some sugars. At first Phillis & Mason

(80a) thought that sugars are accumulated in the transition and companion cells and move from these into the sieve tubes. They discarded this view, reasoning that the process has to pass in both directions as carbohydrates can also be imported into the leaf. Therefore they formulated a new hypothesis, i.e., the substances are partitioned between the tissues depending on their solvent capacity. The relative increase of sugar in different tissues of the leaf should be the same. This new conception has not brought better insight and seems superfluous since the sieve tubes can give off substances to the cytoplasm of other cells at their sieve fields and can obtain them from their companion cells so that there can be movement of substances in both directions. The view that the concentration in the cytoplasm is decisive is fundamental. But it has to be accepted that the cytoplasm below a certain level takes up substances from the sieve tubes and, above this level, the substances are moved eventually by accumulation in the companion cells into the sieve tubes. The data of Roeckl (86), that the osmotic value of the vacuoles of the mesophyll cells is lower than that of the collecting cells and these lower again than those of the sieve tubes, are not at variance with this assumption.

FACTORS WHICH INFLUENCE THE DIRECTION OF THE TRANSPORT

We have discussed the way in which substances are transported in parenchyma and in sieve tubes and now have to consider the question of how the direction of the transport is regulated. Factors depending on the vegetative or reproductive phase of the plant influence the activity of the tissues and the organs (15, 48). When the concentration of substances is increased by synthesis or by mobilisation in a portion of the plant, this can be the cause of transport. This happens, e.g., in leaves during photosynthesis or when substances are mobilised. Auxins may have an influence on the activity of the tissues involved [Stuart (110), Curtis & Clark (40)]. A second possibility is that substances are stored or consumed by a tissue and that transport to this place ensues, e.g., to fruits, growing shoots, storage tissues, etc. Here the decreasing concentration in the cytoplasm may be the cause of the supply by transport.

The view that substances are transported to places where they are utilised is not new. Such places are often called sinks. This need not point to a passive process. On the contrary, by use of energy, synthesis of substances, growth of cells, and storage of materials can be obtained. Apparently there is a competition for foodstuffs in the organism, in which a sequence of dominance obtains, fruits—apical buds—lateral buds—roots (40). Competition also exists between younger and older organs, e.g., in barley (116). Williams (123, 124) mentions active competition between different parts of the plant. Transport between tillers is the result of competition. During their growth they withdraw substances from the rest of the plant. The way in which this competition is effected is obscure.

The growth of an organ or tissue depends on a great number of factors. When salts are used in growth, they are ultimately withdrawn by the root from the medium and then their concentration in the medium can become the limiting factor for the uptake. In this range, growth is dependent on the

concentration of the medium. The law of limiting factors of Blackman and Liebig states that the substance which is present in the lowest concentration limits the growth. If this substance is supplied, growth will be enhanced. It is interesting to know whether the uptake of substances other than that in lowest concentration continues when growth is diminished or whether the uptake of these other substances depends on growth. This problem has been studied for the uptake of salts from the medium.

Helder (50), working with maize, showed that phosphate deficiency gives an inhibition of growth and at the same time of the uptake of other ions used for growth. For this purpose the uptake of nitrate was investigated. The plant had to be saturated with nitrate beforehand. When this was done, nitrate was not absorbed as long as there was no opportunity to absorb phosphate. If phosphate was supplied but nitrate was lacking in the medium, growth was resumed but the nitrate was now furnished from the nitrate stored up in the plant. The result was that when nitrate was supplied there was a very strong uptake of nitrate because it was needed both for the growth as well as for the replacement of the used store.

Circulation systems.—It has been mentioned that there is a circulation system in the plant whereby salts are moved from the medium to the xylem and hence to the transpiring parts or through the rays to the bark and then backward through the phloem to the root. This system opens the possibility that, at a place where salts are used by growth, they are withdrawn from circulation [Alberda (1)]. In the root there must be a supplementation from the medium. It seems necessary that the capacity of this circulation system can vary only within definite limits. Such a circulation system for salts seems to be needed in regard to the micronutrients which have to be supplied in minute quantities to the whole plant. The circulation system enables them to reach a great portion of the plant. Mass flow in the sieve tubes also enables all substances in minimal concentration to reach areas of utilization and storage with a feeble transpiration, such as roots and fruits, where they cannot be transported by the transpiration stream. These substances cannot be moved by concentration gradients over such a distance. Whether there are additional circulation processes in the plant, e.g., for foodstuffs [Münch (73)], or for hormones [Bouillenne (23)], is an interesting problem. It reminds one of the circulation of auxins, which are transported to the tip of a coleoptile as proauxins and then go downwards by polar transport.

LITERATURE CITED

1. Alberda, T., *Rec. trav. botan. nederland.*, **41**, 541-602 (1948-49)
2. Andel, O. M. van, Arisz, W. H., and Helder, R. J., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **53**, 159-71 (1950)
3. Arisz, W. H., *Arch. Rubbercult. Nederland.-Indie*, **17**, 220-41 (1928)
- 3a. Arisz, W. H. (Unpublished data)
4. Arisz, W. H., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **45**, 2-8, 794-801 (1942)
5. Arisz, W. H., *Verslag. Gewone Vergader. Afdeel. Natuurk. Nederland. Akad. Wetenschap.*, **52**, 639-45 (1943)

6. Arisz, W. H., *Verslag. Gewone Vergader. Afdel. Natuurk., Nederland. Akad. Wetenschap.*, **53**, 236-60 (1944)
7. Arisz, W. H., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **48**, 420-46 (1945)
8. Arisz, W. H., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **50**, 1019-32, 1235-45 (1947); **51**, 25-33 (1948)
- 8a. Arisz, W. H., and Heikens, H. S. (Unpublished data)
9. Arisz, W. H., and Berger, E. P. (Unpublished data)
- 9a. Arisz, W. H., Helder, R. J., and Nie, R. van, *J. Exptl. Botany* (In press)
10. Arisz, W. H., and Oudman, J., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **40**, 431-39 (1937)
11. Arisz, W. H., and Oudman, J., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **40**, 440-49 (1937)
12. Arisz, W. H., and Oudman, J., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **41**, 810-19 (1938)
13. Arisz, W. H., and van Dijk, P. J. S., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **42**, 820-31 (1939)
14. Arnon, D. I., Stout, P. R., and Sipos, F., *Am. J. Botany*, **27**, 791-98 (1940)
15. Asana, R. D., *Ann. Botany*, **13**, 237-40 (1949)
16. Bauer, L., *Planta*, **37**, 221-43 (1949-50)
17. Bennett, C. W., *Phytopathology*, **34**, 905-32 (1944)
18. Biddulph, O., *Plant Physiol.*, **15**, 131-36 (1940)
19. Biddulph, O., *Am. J. Botany*, **28**, 348-52 (1941)
20. Biddulph, O., and Markle, J., *Am. J. Botany*, **31**, 65-70 (1944)
21. Bledsoe, R. W., and Harris, H. C., *Plant Physiol.*, **25**, 63-77 (1950)
22. Both, M. P., *Rec. trav. botan. néerland.*, **34**, 1-68 (1937)
23. Bouillenne, R., *Année biol.*, **54**, 597-628 (1950)
- 23a. Brouwer, R. (Unpublished data)
24. Broyer, T. C., *Plant Physiol.*, **25**, 367-76 (1950)
25. Buller, A. H. R., *Researches on Fungi*, **5**, 75-167 (Longmans, Green & Co., New York, N.Y., 416 pp., 1933)
26. Burris, R. H., *Botan. Rev.*, **16**, 150-80 (1950)
27. Burström, H., *Physiol. Plantarum*, **1**, 124-35 (1948)
28. Chen, S. L., *Am. J. Botany*, **38**, 203-11 (1951)
- 28a. Collander, R., and Bärlund, H., *Soc. Sci. Fennica, Commentationes Biol.*, [II]9, 1-13 (1926)
29. Collander, R., and Holmström, A., *Acta Soc. Fauna Flora Fenn.*, **60** (1937)
- 29a. Colwell, R. N., *Translocation in Plants with Special Reference to the Mechanism of Phloem Transport* (Doctoral thesis, Univ. California, 1942); cited by Crafts, A. S., and Lorenz, O. A., in Ref. 38.
- 29b. Colwell, R. N., *Am. J. Botany*, **29**, 798-807 (1942)
30. Cooil, B. J., *Plant Physiol.*, **16**, 61-84 (1941)
31. Crafts, A. S., *Plant Physiol.*, **6**, 1-41 (1931)
32. Crafts, A. S., *Plant Physiol.*, **11**, 63-79 (1936)
33. Crafts, A. S., *Plant Physiol.*, **13**, 791-814 (1938)
34. Crafts, A. S., *Botan. Rev.*, **5**, 471-504 (1939)
35. Crafts, A. S., *Protoplasma*, **33**, 389-98 (1939)
36. Crafts, A. S., *Am. J. Botany*, **26**, 172-77 (1939)
37. Crafts, A. S., *Botan. Rev.*, **17**, 203-84 (1951)
38. Crafts, A. S., and Lorenz, O. A., *Plant Physiol.*, **19**, 326-37 (1944)
39. Curtis, O. F., *The Translocation of Solutes in Plants* (McGraw-Hill Book Co., Inc., New York, N.Y., 273 pp., 1935)

- 39a. Curtis, O. F., and Asai, G. N., *Am. J. Botany*, **26**, 16s (1939)
40. Curtis, O. F., and Clark, D. G., *Introduction to Plant Physiology* (McGraw-Hill Book Co., Inc., New York, N. Y., 705 pp., 1950)
41. Curtis, O. F., and Scofield, H. T., *Am. J. Botany*, **20**, 502-12 (1933)
42. Dawson, R. F., *Plant Physiol.*, **21**, 115-30 (1946)
43. Dhillon, A. S., and Lucas, E. H., *Botan. Gaz.*, **112**, 198-207 (1950)
44. Esau, K., *Botan. Rev.*, **5**, 373-432 (1939)
45. Esau, K., *Botan. Rev.*, **16**, 67-114 (1950)
46. Fischer, H., *Z. Botan.*, **30**, 449-88 (1936-1937)
47. Frey-Wyssling, A., *Jahrb. wiss. Botan.*, **77**, 560-626 (1932)
48. Gregory, F. G., *Ann. Rev. Biochem.*, **6**, 557-78 (1937)
49. Hartt, C. E., *Hawaiian Planters' Record*, **47**, 113-32 (1943)
50. Helder, R. J., *Proc. Koninkl. Nederland Akad. Wetenschap.*, [C]**54**, 275-86 (1951)
51. Hildebrand, E. M., and Curtis O. F., *Science*, **95**, 390 (1942)
52. Höfler, K., *Biol. Generalis*, **19**, 90-113 (1949)
- 52a. Höfler, K., *Anz. Akad. Wiss. Wien. Math.-naturw. Klasse, Denkschr.*, **95**, 99-170 (1918)
53. Huber, B., *Ber. deut. botan. Ges.*, **59**, 181-94 (1941)
54. Huber, B., *Fortschr. Botan.*, **13**, 227-49 (1951)
55. Huber, B., and Kolbe, R. W., *Svensk Botan. Tid.*, **42**, 364-71 (1948)
56. Huber, B., and Rouschal, E., *Ber. deut. botan. Ges.*, **56**, 380-91 (1938)
57. Huber, B., Schmidt, E., and Jahnel, H., *Tharandt. forst. Jahrb.*, **88**, 1017-50 (1937)
58. Hülsbruch, M., *Planta*, **34**, 221-48 (1945)
59. Kent, N. L., *New Phytologist*, **40**, 291-98 (1941)
- 59a. Kok, A. C. A., *Rec. trav. botan. néerland.*, **30**, 23-139 (1932)
60. Leonhardt, H., *Ann. Schädlingssk.*, **16**, 85 (1940)
61. Linder, P. J., Brown, J. W., and Mitchell, J. W., *Botan. Gaz.*, **110**, 628-32 (1949)
62. Lundegårdh, H., *Ann. Roy. Agr. Coll. Sweden*, **16**, 339-71 (1950)
63. Lundegårdh, H., *Physiol. Plantarum*, **3**, 103-51 (1950)
64. Luttkus, K., and Bötticher, R., *Planta*, **29**, 325-40 (1939)
65. Mason, T. G., *Sci. Proc. Roy. Dublin Soc.*, **18**, 195-98 (1926)
- 65a. Mason, T. G., and Maskell, E. J., *Ann. Botany*, **42**, 189-253 (1928)
66. Mason, T. G., Maskell, E. J., and Phillis, E., *Ann. Botany*, **50**, 23-58 (1936)
67. Mason, T. G., and Phillis, E., *Botan. Rev.*, **3**, 47-71 (1937)
68. McAlister, D. F., and Krober, O. A., *Plant Physiol.*, **26**, 525-38 (1951)
69. Michel, E., *Z. angew. Entomol.*, **29**, 243 (1941)
70. Mitchell, J. W., in *Plant Growth Substances*, 141-53 (Skoog, F., Ed., Univ. Wisconsin Press, Madison, Wis., 490 pp., 1951)
71. Mitchell, J. W., and Brown, J. W., *Botan. Gaz.*, **107**, 393-407 (1946)
72. Moore, R., *Am. J. Botany*, **36**, 166-69 (1949)
73. Münch, E., *Die Stoffbewegungen in der Pflanze* (Carl Fischer, Jena, Germany, 234 pp., 1930)
74. Münch, E., *Flora*, **136**, 223-62 (1943)
75. Nie, R. van, Helder, R. J., and Arisz, W. H., *Proc. Koninkl. Nederland Akad. Wetenschap.*, **53**, 567-75 (1950)
76. Oudman, J., *Rec. trav. botan. néerland.*, **33**, 350-433 (1936)
77. Palmquist, E. M., *Am. J. Botany*, **25**, 97-105 (1938)
78. Palmquist, E. M., *Am. J. Botany*, **26**, 665-67 (1939)
79. Pfeiffer, M., *Planta*, **19**, 272-78 (1933)

80. Pfeiffer, M., *Flora*, **132**, 1-47 (1937)
- 80a. Phillis, E., and Mason, T. G., *Ann. Botany*, **47**, 585-634 (1933)
81. Rabideau, G. S., and Burr, G. O., *Am. J. Botany*, **32**, 349-56 (1945)
82. Reed, H. S., and Haas, A. R. C., *Calif. Agr. Expt. Sta. Tech. Paper*, No. 4, 1-32, (1923)
83. Rice, E. L., *Botan. Gaz.*, **109**, 301-14 (1948)
84. Robertson, R. N., *Proc. Linnean Soc. N. S. Wales*, **75**, 1-20 (1950)
85. Robertson, R. N., *Ann. Rev. Plant Physiol.*, **2**, 1-24 (1951)
86. Roeckl, B., *Planta*, **36**, 530-50 (1949)
87. Rohrbaugh, L. M., and Rice, E. L., *Botan. Gaz.*, **111**, 85-89 (1950)
88. Rouschal, E., *Flora*, **135**, 135-200 (1941)
89. Rouschal, E., and Strugger, S., *Ber. deut. botan. Ges.*, **48**, 50-69 (1940)
90. Ruhland, W., *Jahrb. wiss. Botan.*, **55**, 409-98 (1915)
91. Said, H., *Nature*, **162**, 496 (1948)
92. Sandström, B., *Physiol. Plantarum*, **3**, 496-505 (1950)
93. Schumacher, A., *Planta*, **35**, 642-700 (1948)
94. Schumacher, W., *Jahrb. wiss. Botan.*, **73**, 770-823 (1930)
- 94a. Schumacher, W., *Jahrb. wiss. Botan.*, **75**, 581-608 (1931)
95. Schumacher, W., *Jahrb. wiss. Botan.*, **77**, 685-732 (1933)
96. Schumacher, W., *Jahrb. wiss. Botan.*, **80**, 74-91 (1934)
97. Schumacher, W., *Jahrb. wiss. Botan.*, **82**, 507-33 (1936)
98. Schumacher, W., *Jahrb. wiss. Botan.*, **85**, 422-49 (1937)
99. Schumacher, W., *Jahrb. wiss. Botan.*, **88**, 545-53 (1939)
100. Schumacher, W., *Naturwissenschaften*, **34**, 176-79 (1947)
101. Schumacher, W., *Planta*, **37**, 626-34 (1950)
102. Schumacher, W., and Halbgueth, W., *Jahrb. wiss. Botan.*, **87**, 324-55 (1939)
103. Shapiro, B., *Biochem. J.*, **41**, 151-54 (1947)
104. Steinbach, H. B., *Ann. Rev. Plant Physiol.*, **2**, 323-42 (1951)
105. Stout, M., *Botan. Gaz.*, **107**, 86-95 (1945)
106. Stout, P. R., and Hoagland, D. R., *Am. J. Botany*, **26**, 320-24 (1939)
107. Street, H. E., and Lowe, J. S., *Ann. Botany*, **14**, 307-29 (1950)
108. Strugger, S., *Flora*, **132**, 253-304 (1938)
109. Strugger, S., *Praktikum der Zell- und Gewebephysiologie der Pflanze*, 2nd Ed. (Springer-Verlag, Berlin, Germany, 225 pp., 1949)
110. Stuart, N. W., *Botan. Gaz.*, **100**, 298-311 (1938-39)
111. Tammes, P. M. L., *Rec. trav. botan. néerland.*, **30**, 514-36 (1933)
112. Tammes, P. M. L., *Rec. trav. botan. néerland.*, **41**, 737-39 (1949)
113. Tammes, P. M. L., *Proc. Koninkl. Nederland Akad. Wetenschap.*, [C]**54**, 30-31 (1951)
114. Tammes, P. M. L. (Personal communication)
115. Tingley, M. A., *Am. J. Botany*, **31**, 30-38 (1944)
116. Walkley, J., *New Phytologist*, **39**, 362-69 (1940)
117. Weeks, D. C., and Robertson, R. N., *Australian J. Sci. Research*, [B]**3**, 487-500 (1950)
118. Weintraub, R. L., and Brown, J. W., *Plant Physiol.*, **25**, 140-49 (1950)
119. Went, F. W., *Jahrb. wiss. Botan.*, **76**, 528-57 (1932)
120. Went, F. W., and Carter, N., *Am. J. Botany*, **35**, 95-106 (1948)
121. Wiersum, L. K., *Rec. trav. botan. néerland.*, **41**, 1-80 (1948-49)
122. Wiersum, L. K., *Plant and Soil*, **3**, 160-69 (1951)
123. Williams, R. F., *Australian J. Exptl. Biol. Med. Sci.*, **16**, 65-83 (1938)
124. Williams, R. F., *Australian J. Sci. Research*, [B]**1**, 333-61 (1948)

LEAF PROTEINS^{1,2}

BY S. G. WILDMAN AND ANDRE T. JAGENDORF³

Division of Botany, University of California, Los Angeles, California

INTRODUCTION

Present interest in the chemistry and metabolism of proteins is widespread, and understandable because of the fundamental role that these molecules occupy in most biological phenomena. The geneticist is interested in the self-duplication of nucleoproteins, the enzymologist in the ability of proteins to catalyze chemical reactions, and the chemist in the orderliness by which such huge molecules are fashioned. As a consequence, the field of protein chemistry and metabolism has become so vast and complicated that it is now extremely difficult for the average investigator to keep abreast of the expanding literature and to avoid extreme specialization in his own branch of protein investigation. Consider, for example, the case of the leaf proteins. The enzymologist may have a vital interest in the leaf as a desirable source of the enzyme he proposes to investigate, but in the main his concern is only for a particular protein or proteins often present in minute amounts; hence, he may view the remaining proteins of the leaf as unwelcome contaminants of the pure products he is striving to produce. Similarly, the virologist is interested in the leaf as a source of abnormal protein which arises as the result of virus infection, but again his interest in the normal complement of leaf proteins is somewhat incidental to the problem of keeping them from contaminating his product. Likewise, the photosynthesis workers have largely confined their attention to the pigmented structures of the leaf whose protein components are undoubtedly responsible for a large part of the photosynthetic reactions in question.

In this review, we should like to place our emphasis on the extraction and nature of the entire complement of proteins to be found in the protoplasm of leaf cells, since it would seem that the solution of many future problems in plant physiology and biochemistry may depend upon exact knowledge of the types of proteins which are encountered in leaf protoplasm and the means available for their isolation and characterization. The composition of leaf proteins, together with their metabolic and physiological behavior, has formed the subject of numerous reviews, some of them quite recent [Chibnall (11); Lugg (30); Vickery (56); Bonner (6); McCalla (32); McKee (33); Wood (63); Steward & Thompson (52)]. To avoid needless

¹ The survey of the literature pertaining to this review was concluded in December, 1951.

² Some of the work reported here was facilitated by a financial grant from the Atomic Energy Commission. We wish to thank Mr. Luther Eggman and Dr. Guy Camus for allowing us to quote some of their unpublished experiments.

³ Merck Post-doctoral Fellow, 1951-1952.

repetition, we intend only to examine the field as we find it today, indicate some of the directions that future research might, in our opinion, profitably pursue and stress the urgent need for the development of quantitative methods for the more complete separation of the components of leaf protoplasm. We are convinced that the development of quantitative methods is a prerequisite to a satisfactory correlation of the structure and metabolic function of proteins at the subcellular level.

Problems concerned with the extraction of leaf proteins.—The typical palisade or spongy parenchyma cell of the leaf is constructed of a rather heavy cellulose cell wall surrounding an extremely thin layer of protoplasm which encloses a large vacuole. One semipermeable membrane separates the protoplasm from the cell wall while another membrane isolates the protoplasm from the vacuole. The typical cell contains a single nucleus, perhaps as many as 50 disc-shaped chloroplasts which are composed of grana imbedded in a microscopically invisible stroma, and numerous smaller structures some of which have been classified as mitochondria [Newcomer (38)]. All of these structures can be seen with the aid of a light microscope, and it is estimated that up to 45 per cent of the total protein nitrogen of the leaf may be found in such particulate structures [Hanson (24), Granick (21, 22)]. The remaining protein nitrogen is present in the soluble protoplasmic matrix bathing the particulate matter.

Chibnall's classical work in this field has shown that the cell wall acts as an ultrafilter in preventing the passage of leaf proteins (11). Destruction of the semipermeable membranes could be accomplished by ether cytolysis without rupture of the cell walls, after which nearly all of the low molecular weight constituents of the protoplasm and vacuole could be extruded through the pores of the cell wall by moderate pressure. Essentially no protein could be found in the juice. The fact that the intact cell wall prevents the passage of high molecular weight substances presents therefore a serious problem in the extraction of leaf proteins, viz., how to rupture the cell walls and release the proteins without seriously altering the particulate structures of protoplasm.

Animal biochemists [Potter *et al.* (40), Hogeboom (27), Claude (12)], have found nuclei and mitochondria to be extremely fragile and susceptible to fragmentation with the release of some of their proteins in soluble condition, even with the gentle treatments required to disrupt animal cells, which lack cell walls. Chloroplasts are notoriously fragile structures [Neish (37), Menke (35)] which are broken into large chloroplast fragments and smaller grana by most techniques designed to accomplish the disruption of a large number of the total cells of the leaf. At best, extraction methods must represent a compromise between the desire of the investigator to rupture as many cells as possible and thus approach a total protein extraction of the leaf, and the necessity to maintain protoplasmic materials in as nearly unchanged a state as possible.

A second major problem in the extraction of leaf proteins is founded on

the fact that a large part of the total volume of the leaf cell is occupied by the vacuole, the contents of which are often quite acid. When the membrane separating the protoplasm and vacuole is destroyed as in grinding operations, or in freezing and thawing, two adverse conditions are encountered: (a) the protoplasm, and hence the proteins, undergoes a relatively enormous dilution, and (b) the proteins may enter an environment the acid conditions of which can cause the coagulation of the soluble proteins and aggregation of the particulate structures. If the normally soluble cytoplasmic proteins are denatured and flocculated by acid vacuolar juice, the apparent yield of cytoplasmic proteins will be low, as a major portion of these collect with the sedimentable particles and cell wall residues during subsequent fractionation procedures.

An example where extremely low yields of soluble cytoplasmic proteins can probably be attributed to denaturation in the presence of vacuolar juice rather than failure to rupture the leaf cells and release the proteins is that of a recent investigation [Meneghini & Delwiche (34)] on the incorporation of N¹⁵ into tobacco mosaic virus compared to the normal cytoplasmic proteins of tobacco leaves. It is possible to compare their extraction results with work of a similar nature [Wildman *et al.* (61)], as shown in Table I. There is agreement in the amount of virus which can be extracted from

TABLE I

A COMPARISON OF THE AMOUNT OF VIRUS AND CYTOPLASMIC PROTEIN EXTRACTED FROM TOBACCO LEAVES IN THE PRESENCE OF BUFFER OF DIFFERENT STRENGTH*

Days after infection	Mg. virus		Mg. cytoplasmic protein	
	Ref. (34)	Ref. (61)	Ref. (34)	Ref. (61)
5	—	0	—	8.7
6	0.24	—	2.5	—
8	1.65	—	0.65	—
10	2.40	—	1.1	—
12	—	1.92	—	7.7
17	—	3.86	—	5.8
35	3.40	—	1.1	—

* See text for discussion of the results. All values computed on a gram fresh weight basis.

infected leaves as a function of time, indicating that the leaf cells were about equally ruptured, but there exists a great disparity in the quantity of cytoplasmic protein extracted. Presumably, the loss in cytoplasmic protein can be accounted for through the use of too weak a buffer (0.1 M), resulting in acid-coagulated protein which was discarded either with the cell wall residues or with the particulate matter. Virus protein, on the other hand, does not flocculate in the presence of tobacco leaf juice. Obviously,

it would be highly desirable in this type of investigation to use samples more nearly representative of all of the proteins in cytoplasm. Other virus workers have also experienced very low yields of normal cytoplasmic proteins [Wyckoff *et al.* (51), Takahashi (55)]. The method used primarily for the extraction of virus, consists of first freezing entire leaves and then adding a dry buffer salt to the material during thawing and mincing. Thus, a neutral juice is obtained which however, contains little protein other than virus protein. When this method is applied to uninfected leaves as a means of extracting normal proteins, the probability is that the time required for solution of the buffer salt in plant juice prior to its penetration through the cell wall into the protoplasm is sufficiently long to have permitted the coagulation and aggregation of the proteins by the vacuolar juice before neutralization. The acid conditions arising from vacuolar juice can be largely controlled by the use of strong buffers during grinding, but there is at present no convenient method which avoids or minimizes the effect of dilution on the leaf protoplasm.

Various means have been used to rupture the cell wall preliminary to the extraction of the protoplasm. Crook (15) has investigated the use of the runner or roller mill; Wildman & Bonner (57) have compared the efficiency of the blender and the colloid mill. Obviously, the choice of grinding device will depend upon the type of investigation. Where gentle procedures are called for, as in the preparation of intact chloroplasts, sand in a mortar and pestle may provide the largest yield of unaltered chloroplasts. In the extraction of soluble proteins, the colloid or runner mill affords a simple method whereby large yields of protein may be obtained, although the high shearing force generated by the mill generally breaks down the chloroplasts and presumably the nucleus into much smaller fragments.

Cell walls and unbroken cells can be removed by filtration or low speed centrifugation after rupture of the cell wall. Where large quantities of leaf material are to be processed, a basket centrifuge lined with sharkskin filter paper provides a convenient method for separating cell walls from protoplasm (57).

PROTEINS IN PARTICULATE MATTER

General.—For convenience, the particulate matter will be defined as the structures in leaf protoplasm which are visible in the organized cell with the aid of the ordinary light microscope, and therefore have dimensions in excess of about 0.5μ . In the main, the particulate matter consists of nuclei, chloroplasts, and mitochondria, although starch grains and materials like calcium oxalate crystals also fall under this classification. Consisting of particles of large mass relative to the other constituents of protoplasm, the particulate matter can be compacted as a sediment by centrifuging, and centrifugal forces of about 20,000 g applied for about 1 hr. are sufficient to remove all microscopically visible particles from a neutral protoplasmic extract (57, 60). Although the microscope clearly reveals particles in leaf

protoplasm which differ greatly in shape and dimensions, ways and means for separating each class of particles are not available. In contrast, the animal biochemist is now in the fortunate position of being able to purify nuclei and mitochondria in almost quantitative yield. The only particles which have been prepared from leaves in a relatively purified condition are the chloroplasts.

The isolation of intact chloroplasts.—Chloroplasts behave as particles surrounded by a semipermeable membrane (21, 35), and hence are prone to burst in hypotonic solutions. Thus, it is necessary to disrupt the cells by very gentle means in the presence of a solution which will prevent bursting or plasmolysis of the particles. The method used in the preparation of whole chloroplasts [Granick (21); Neish (37); Hanson (24); Bot (7)] is, in general, to grind the leaf material for very short periods of time in the presence of hypertonic sucrose solutions. After removal of the cell wall debris, intact chloroplasts are sedimented by low speeds leaving the smaller particles in the supernatant liquid. The chloroplasts are resuspended and the process repeated. The yield of intact chloroplasts has been quite low since the nature of the separation process results in fragmentation. Because of the difficulties in preparation together with the low yields of intact chloroplasts, most investigations dealing with the chemical nature of the particulate structures have been concerned with fragments of chloroplasts, and there is practically no information on chloroplasts in nearly pure and unaltered state as judged by cytological observation. It is clear that much more needs to be done in this field of plant protein chemistry.

The protein composition of chloroplast fragments.—Beginning with Chibnall, investigations have been pursued concerning the protein composition of fragments of chloroplasts. Depending upon the vigor of grinding, a range of particle sizes is obtained from intact particles down to grana. Although very low centrifugal forces are capable of separating intact chloroplasts from cell-free preparations, intense centrifugal fields are required to sediment all of the fragmented chloroplastic material in a nonaggregated state (57). Centrifugation of such chloroplastic material at moderate speeds may be aided by prior chemical or physical treatments causing aggregation of the fragments. Acidification (35), ammonium sulfate (35, 57), calcium ions (14), and alternate freezing and thawing (14, 61) are methods that have been used. There are three possible difficulties to be considered in the use of such methods for obtaining chloroplastic material for chemical analysis.

The first possibility is that some of the material originally present in the intact chloroplast will be lost as soluble protein when fragmentation occurs. Secondly, aggregation may also cause the denaturation and precipitation of normally soluble cytoplasmic proteins so that they are centrifuged down with the chloroplastic matter. For example, Comar (14) reports that spinach chloroplast fragments which were aggregated by calcium ions or freezing and thawing had a higher protein/lipoid ratio than similar preparations which were collected by centrifuging in the presence of distilled

water. These results suggest that coagulation of cytoplasmic proteins led to their incorporation into the sedimentable particulate matter with consequent enrichment of protein compared to lipid. However, it has been our experience that aggregation of tobacco leaf chloroplast fragments by freezing and thawing can be accomplished without appreciable coagulation of the cytoplasmic protein providing the extract is not allowed to become more acid than pH 6.4.

The third and most serious difficulty resides in the wide distribution of particle sizes derived from grinding the chloroplasts, which virtually precludes the possibility of separating other particles such as mitochondria or nuclear fragments from the chloroplastic matter. On this account, it is doubtful if chloroplastic material has yet been prepared which can be considered uncontaminated by other particulate matter of protoplasm. Progress in this direction will be seriously delayed until methods are devised which will prevent or minimize fragmentation and subsequent intermingling of larger particles, or which will permit the selective aggregation of the chloroplast or nuclear fragments without affecting other particles of the protoplasm.

The quantity of chloroplastic material in the leaf cells has been investigated through the isolation of chloroplastic material and determination of the chlorophyll/nitrogen ratio. From a knowledge of the total chlorophyll of the leaf, the total chloroplastic nitrogen can be calculated. In this manner it was found for tomato and tobacco (21, 22), clover (37), and oat leaves (20) that between 30 and 40 per cent of the total leaf nitrogen is contained in the chloroplasts, and 35 to 45 per cent of the spinach leaf nitrogen is found in the chloroplasts (35). Thirty-two to 36 per cent of the total leaf protein is found in the chloroplasts of *Phalaris tuberosa* (24) and 35 to 40 or 35 to 45 per cent, respectively, for those of Sudan grass (25), tomato and tobacco (22).

Chloroplastic preparations have been found to contain proteins, lipoidal material in addition to the chlorophyll and yellow pigments, and ash constituents. The proportions in which these constituents are found are listed in Table II. The composition of chloroplastic material, together with the available evidence on the state of binding of ash constituents and pigments

TABLE II
COMPOSITION OF CHLOROPLASTIC MATERIAL

Plant	Per cent protein	Per cent lipids	Per cent pigments	Per cent ash	Reference
Spinach	48	31	—	18	Menke (35)
Spinach	40	25	—	17	Chibnall (11)
Spinach	54	34	5	7	Comar (14)
Spinach	48-54	26-32	4-6	—	Bot (7)
<i>Lathyrus odoratus</i>	33-50	18-30	4-6	—	Bot (7)
Clover	50	22	—	—	Neish (37)

in the chloroplast, has been reviewed in great detail by Rabinowitch (42). He also weighs the evidence for the presence of a chlorophyll-protein complex in chloroplasts. In another review, Aronoff (3) takes up the various enzymatic activities which have been described for chloroplastic material. It is of particular interest that some of the oxidative mechanisms of leaf protoplasm have been found in the particulate matter [Laties (29); DuBuy, Woods & Lackey (17)], but it is still an open question as to whether the activity is a property of the chloroplastic matter itself, or of non-green particles which contaminate the chloroplastic matter. The identification of cytochrome pigments as a constituent of leaves [Hill & Scarisbrick (26)] suggests that chemically definable mitochondrial particles may exist in leaves, and future work may result in their separation from chloroplastic matter. The importance of devising methods for the separation of the various cellular particles in a reasonably homogeneous state, and the careful characterization of such preparations both from the cytological and chemical point-of-view, can scarcely be overemphasized. The startling advances in understanding the physiology of animal tissue which have resulted from the ability to separate and work with individual structural components of protoplasm provide an adequate illustration of this point [Claude (12)].

In regard to the protein contained in the chloroplastic material itself, the question of concern is whether the protein is primarily of one kind, perhaps combined with chlorophyll, or a mixture of various proteins each of which might have a different function in the metabolism of the intact chloroplast. Various attempts have been made to prepare soluble proteins from chloroplastic material (2, 46-50, 53, 54). Strenuous grinding of chloroplastic material in the presence of water or buffer has produced stable suspensions of green liquid with no particles which could be detected by the light microscope [Stoll & Weidemann (53)]. However, particles could be detected by means of dark-field illumination and moderate centrifugal fields were sufficient to sediment the green material suggesting that the chloroplastic material had not been greatly reduced in size [Smith (46)]. Furthermore, examination of the stable suspensions in the analytical centrifuge proved them to be polydisperse, and the fact that the same proportion of pigments, lipid, and protein was found in the suspension as in the original chloroplastic material suggests that reduction in size of the particles occurred without the release of a specific chlorophyll-protein.

Smith (47, 48) and Smith & Pickels (49, 50) have investigated the use of surface active agents as a means of dispersing chloroplastic material. While it is possible to prepare green solutions, these chemicals either result in the complexing of the surface active agent with the chloroplastic material, or the cleavage of chlorophyll from the protein, or the cleavage of magnesium from the pigments. Stoll, Wiedemann & Rügger (54), however, have found that treatment of chloroplastic material with sodium cholate resulted in a green solution which was electrophoretically homogeneous, monodisperse, and which had a molecular weight of about five million. Price & Wyckoff (41)

have obtained a green protein from plant leaves without the use of sodium cholate which is also of about five million molecular weight.

In view of the ease with which the proteins of animal particulate matter can be solubilized by mechanical treatment, it is somewhat surprising that the protein contained in chloroplastic material is so resistant to dispersion. This peculiarity may be related to the high lipid content of chloroplastic material such that if lipids were bound to protein, the whole complex would take on a highly hydrophobic character with little tendency for the protein molecules to separate from the lipid and go into colloidal solution. Milner, Koenig & Lawrence (36) have partially solubilized chloroplastic material by forcing it through a small orifice under high pressure, the shearing force causing fragmentation of the particles to a smaller size. It is interesting to note that the ability of their preparations to carry out the Hill reaction was dependent upon the state of aggregation, and more particularly on the lipid content of the dispersions. The material after passage through the orifice was essentially inactive, but when aggregated by the addition of salt, the activity was largely restored. However, if the lipid content was lowered by solvent extraction, neither recovery of activity nor aggregation of the particles could be induced by the presence of salt. If we are to believe that the Hill reaction is mediated by an enzyme-protein, it appears that lipid is an essential component for the activity of the protein.

Mitochondria.—DuBuy, Woods & Lackey (17) have investigated the metabolism of a mitochondrial fraction isolated from tobacco leaves. In their procedure, a brownish layer is deposited on top of a green layer of chloroplastic material when leaf homogenates are centrifuged. Upon resuspension of the brown material the preparation is able to perform some of the oxidative reactions characteristic of animal mitochondria. Since the grinding is accomplished in the presence of an unbuffered sucrose solution, it is possible that some of the material in the brownish layer arises from coagulation of normally soluble cytoplasmic proteins under the influence of the acid vacuolar juice. Since it was also stated that the mitochondrial preparations were contaminated with chloroplastic matter, it is difficult to assess the purity of the preparations. Staining with Janus green, a reagent commonly employed in the identification of animal mitochondria, would be a useful further criterion for the presence of mitochondria in plant extracts.

In summary, the preparation of nuclei, chloroplasts, and mitochondria in reasonably pure condition from leaf tissue has lagged far behind the preparation and purification of comparable structures from animal protoplasm. While the unique architecture of the plant cell is responsible for some of the major difficulties in the fractionation of particulate components, nevertheless the fact that nuclei and chloroplasts have been freed from the cell in a condition microscopically identical to that seen in organized protoplasm encourages us to believe that concerted effort and improved methods will lead to the further fractionation and purification of the elements of the particulate matter.

THE CYTOPLASMIC PROTEINS

General properties.—The proteins which remain in solution after removal of the particulate matter are usually referred to as cytoplasmic proteins, although this is a somewhat unfortunate designation since the cytologist defines cytoplasm as consisting of everything in the cell except the nucleus and the cell wall. A better term would perhaps be the soluble proteins of protoplasm, but such a term does not convey the notion that these are proteins which bathe particulate structures and are contained in the thin layer of protoplasm which is separated from the vacuole. Although definitive proof is lacking, the impression is also gained that these proteins as extracted correspond to their character in protoplasm and are not degradation products derived through the breakdown and solubilization of the particulate matter.

In contrast to the difficulty in working with the proteins of the particulate matter, the cytoplasmic proteins can be examined by conventional methods of protein analysis such as electrophoresis and analytical ultracentrifugation, and it is largely from this type of analysis that our information on the protein makeup of cytoplasm is derived. A large portion of the total protein nitrogen of leaf protoplasm is concentrated in the soluble cytoplasmic proteins (11, 35, 57).

The preparation of cytoplasmic proteins.—The chief problems associated with the preparation of the cytoplasmic proteins are to free them completely from the finer particles of the protoplasm, and to prevent the precipitation of the proteins during extraction since they are unusually sensitive to even mildly acid conditions. Chibnall [(11), p. 142], reports the pH of extracted leaf sap to vary from 5.4 to 6.8 depending upon the species of plant. Most saps have a pH of 5.5 to 5.6, as is that from mature tobacco leaves. Eggman *et al.* (18) have found that if a 1 per cent solution of tobacco cytoplasmic proteins is subjected to pH 5.5, more than 50 per cent of the total protein will flocculate to the extent that the protein will be deposited as a sediment by low speed centrifugation. Thus, unless special precautions are taken to keep the cytoplasmic proteins neutral, there is great danger that they will be precipitated and discarded with either the cell wall residues or along with the particulate matter.

In our experience, the fractionation scheme illustrated by Figure 1 provides a method which not only results in large yields of the cytoplasmic proteins but also leads to reasonably reproducible preparations by various criteria when leaves of approximately the same age are used (45). Fine grinding of the leaf tissue is achieved by colloid milling, followed by removal of the cell walls by filtration. Wide departures from neutrality can be avoided with most leaf tissues if two parts of leaves are ground in the presence of 1 part of pH 7, M/2 buffer. The yield of protein is essentially 90 per cent complete with the first extraction (57).

The cell-free protoplasm can be further fractionated by spinning at high speed in a preparative ultracentrifuge. A green and white sediment is deposited in a tightly-packed condition leaving a sparkling clear, nonopalescent

amber to brown supernatant solution containing the cytoplasmic proteins. If a high speed centrifuge is not available, it is possible to remove the particulate matter at lower speeds if the cell-free protoplasm is first frozen

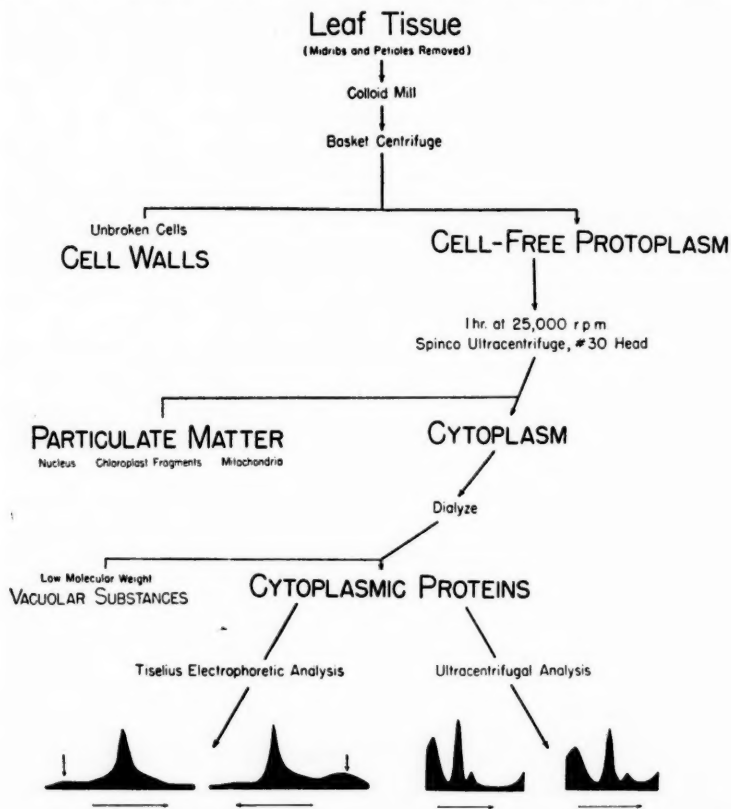


FIG. 1. A scheme for the preparation of leaf cytoplasmic proteins. Horizontal arrows indicate direction of protein migration; vertical, position of starting boundaries.

[For discussion of the general principles of electrophoresis and ultracentrifugation, see Gray (23, 23a). A lucid but more technical treatment of electrophoresis is given by Alberty (1). The ultracentrifuge is considered in some detail by Kabat & Mayer (28).]

for 24 hr. or longer and then thawed before centrifuging. Contrary to the experience of others [Pirie (39)], we have been unable to detect any change in the solubility or in the physical behavior of the cytoplasmic proteins which have been frozen in neutral solutions. It is also our experience that

preparations of cell-free protoplasm as well as the cytoplasmic proteins can be stored in the frozen condition for several weeks and still return completely to solution after being thawed.

The color of cytoplasmic protein solutions.—Prepared according to the above scheme of analysis, solutions of cytoplasmic proteins, 1 per cent or more by weight, may be obtained. Such solutions are always brownish colored, the intensity and shade of the color varying with the species of plant leaves extracted [Campbell, (8)]. It is not clear whether this color is an inherent property of the proteins or is a result of oxidative discoloration accelerated by the extraction process or a combination of both. The color of some protein solutions may indeed darken upon standing, even at low temperatures; in other leaves, oxidative darkening is not so evident. The color is not removed during prolonged dialysis, and for the most part precipitates with the proteins during isoelectric or trichloroacetic acid precipitation, and has been observed to move with the protein boundaries during electrophoresis and ultracentrifugation. At the very least, it can be said that the color moiety is either a part of the protein or else extremely tightly adsorbed to it.

Attempts have been made [Woods & DuBuy (65)] to prevent oxidative discoloration of tobacco leaf proteins by including 0.5 per cent thiourea as an antioxidant in the extraction medium. Such preparations are less dark. However, Camus (9) found that grinding tobacco leaves in the presence of 0.5 per cent thiourea completely eliminated the ultracentrifugal peak due to Fraction I protein which normally accounts for about 50 per cent of the total cytoplasmic protein preparation. The actual cause of this disappearance of protein in the presence of thiourea has not been determined, but is most likely related to the efficient action of thiourea as a protein denaturant. Campbell (8) has attempted to use other antioxidants, less likely to act as protein denaturants, to prevent oxidative discoloration of corn and sugar beet cytoplasmic proteins, but the treatments failed to affect an appreciable reduction in the color of the final preparations.

The composition of the cytoplasmic proteins of dicotyledonous leaves.—Since investigations on the amino acid composition of leaf protein preparations have been recently and thoroughly reviewed by Lugg (30), other physical and chemical properties of the cytoplasmic proteins will be considered in this section. Frampton & Takahashi (19) obtained evidence that the cytoplasmic proteins of tobacco leaves migrated as three components in the electrophoresis apparatus, the fastest constituting but a small portion of the total area of the scanning pattern. A component of intermediate mobility occupied more than 50 per cent of the total pattern. A third component, also comprising a large part of the total area, moved at a very slow rate, if at all, and may have been an exaggeration of the starting boundaries which remain present during electrophoresis. Frampton & Takahashi used phosphate buffers which were later shown to result in unusually large starting boundary anomalies with tobacco cytoplasmic proteins (61). Therefore, it seems reasonable to interpret Frampton & Takahashi's pat-

terns as indicating the migration of nearly all of the tobacco cytoplasmic proteins as a single boundary. Their results with pea-bean leaves were closely similar to those of tobacco except for the absence of the first, minor component.

More extensive electrophoretic investigations of cytoplasmic proteins extracted from numerous genera and species of leaves show that in each instance, more than 60 per cent of the total protein migrates as a single boundary (8, 58). The patterns at the left bottom of Figure 1 are typical, although the number of minor components which resolve during electrophoresis may vary from one to five depending upon the source of cytoplasmic proteins. Electrophoresis, thus, gives some indication that a large part of the total cytoplasmic proteins consists of one kind of protein with respect to electrical charge.

Further evidence that the cytoplasmic proteins of dicotyledonous leaves consists in large part of a single species of protein comes from experiments with the analytical ultracentrifuge [Singer *et al.* (45)], in which resolution of proteins in a mixture depends upon differences in mass rather than upon electrical charge as in electrophoresis. The scanning patterns for tobacco cytoplasmic proteins shown at the bottom right side of Figure 1 are again typical of those encountered when the cytoplasmic proteins from other leaves are analyzed in the ultracentrifuge. The various cytoplasmic proteins are generally resolved into three peaks, two of which make up about 90 per cent of the total area of the pattern. The third, minor component, when present, moves in the gravitational field as the heaviest component with a sedimentation constant of about 24 Svedberg units. There is some reason to believe, as will be shown later, that the 24S component, when present, is formed by aggregation of the cytoplasmic proteins during preparation.

The middle component, comprising from 23 to 50 per cent of the total area of the pattern depending upon the cytoplasm examined, is characterized by a remarkably symmetrical peak suggesting a protein of great homogeneity with respect to molecular weight. This protein, called Fraction I protein, has a sedimentation constant of around 18S in the cytoplasm of all the species investigated and appropriate calculations reveal Fraction I protein to have a molecular weight close to 600,000.

The trailing component making up the third peak, having the least mass and a mean sedimentation constant of 4S, lacks symmetry and must be considered heterogeneous; it apparently represents all of the other proteins of the cytoplasm. For convenience, this mixture of proteins is collectively referred to as Fraction II.

Thus, the picture that emerges from the electrophoretic and ultracentrifugal examination of the cytoplasm a variety of dicotyledonous plants is that the protein mixtures are remarkably similar in general composition, and that each is characterized by the presence of a homogeneous, high-molecular weight, main protein component which comprises up to 50 per cent of the total protein mixture.

The cytoplasmic proteins of monocotyledonous leaves.—When the cyto-

plasmic proteins extracted from corn and oat leaves were analyzed by electrophoresis and ultracentrifugation (8), they differed markedly from dicotyledonous cytoplasmic proteins in that no evidence could be obtained for the presence of a protein corresponding to Fraction I protein. During electrophoresis, monocot cytoplasmic proteins migrate as broad, diffuse mounds rather than sharp peaks. In the ultracentrifuge, only a heterogeneous mixture of proteins having a mean sedimentation constant of about 4S could be identified. While these results must be considered preliminary since they involve only two genera of monocots, nevertheless they suggest that one of the striking biochemical differences between monocots and dicots may reside in the cytoplasmic proteins, and it will be an important future task to assess the significance of this difference.

The isolation of fraction I protein.—Two methods have been used in the separation of Fraction I protein from other proteins of cytoplasm. The first involves selective precipitation of the protein with ammonium sulfate (57, 60), and the second involves preparative ultracentrifugation (39, 18).

Appreciable precipitation of cytoplasmic proteins from a 1 per cent neutral solution occurs at about 35 per cent saturation with ammonium sulfate. Under these conditions, 89 per cent of the cytoplasmic proteins of *Nicotiana glutinosa*, 88 per cent of *N. tabacum*, 75 per cent of spinach, 78 per cent of cucumber, and 70 per cent of tomato can be precipitated and then completely redissolved in neutral buffer. The proteins of *Xanthium* are exceptional in that they will not redissolve in buffer after ammonium sulfate precipitation. The redissolved proteins, when examined in the ultracentrifuge consist of about 80 per cent Fraction I protein, and 20 per cent of smaller molecular weight proteins. Further precipitation with ammonium sulfate has failed to affect this ratio of proteins appreciably, and it appears that a clean-cut separation of Fraction I protein from Fraction II cannot be achieved by this method, although a substantial enrichment of Fraction I protein is obtained. A further disadvantage is that ammonium sulfate precipitation appears to cause the dissociation of organic phosphorus which initially precipitated with the protein in the presence of trichloroacetic acid.

As far as we are aware no serious attempts have yet been made to use precipitating agents of lower dielectric constant than ammonium sulfate solutions. Alcohol, when employed at temperatures below zero, can be used for the selective precipitation of certain protein components in plasma [Cohn *et al.* (13)], and it would be of interest to investigate the use of alcohol and other agents in the fractionation of the cytoplasmic proteins of leaves.

The proteins of cytoplasm can be flocculated by other means such as isoelectric precipitation (11, 62); however, in general, such methods produce precipitates which fail to redissolve in neutral buffer, but redissolve only in the presence of strong bases or acids. Because of the change in solubility, such preparations must be considered partially, if not completely, denatured.

The second method used in the preparation of Fraction I proteins in-

volves preparative ultracentrifugation. The development of the method is derived from information on the behavior of cytoplasmic proteins gained in the analytical ultracentrifuge. Because it is heavier than the Fraction II proteins, it is possible to concentrate Fraction I protein by applying forces equivalent to those which cause the migration of the protein in the analytical centrifuge. It should be pointed out that the degree of separation of the two protein fractions not only depends upon their sedimentation constants but also upon the proportion of each found in the original mixture. The amounts of each which will be deposited as a pellet will be approximated by the formula:

$$\frac{\text{Per cent Fraction I in mixture}}{\text{Per cent Fraction II in mixture}} \times \frac{\text{sedimentation constant of Fraction I}}{\text{sedimentation constant of Fraction II}}$$

Since area measurements indicate about equal parts of Fraction I and Fraction II in tobacco cytoplasm, and the sedimentation constants are 18S and 4S respectively, the pellet would be expected to contain 18 parts of Fraction I to 4 parts Fraction II, or a Fraction I preparation of about 80 per cent purity. If the centrifuging were repeated, a pellet containing 18 parts Fraction I to 1 part Fraction II would yield a Fraction I preparation of 95 per cent purity. If a smaller proportion of Fraction I protein were present initially, it can be seen that many more cycles of centrifugation would be required for comparable purification.

The expected purification of Tobacco Fraction I protein is achieved by centrifuging a 1 per cent cytoplasmic protein solution for 3 hr. at 40,000 r.p.m. (approximate centrifugal force, 140,000 g.) in the size 40 head of a Spinco Model L centrifuge. After two cycles of centrifugation, about 20 per cent of the total starting protein is recovered from the completely redissolved pellet. This, in the analytical ultracentrifuge, is seen to consist almost entirely of 18S and 24S components, with not more than 5 per cent Fraction II present.

Two reasons are offered in support of the idea that the 24S component is an aggregate of the 18S component. One is that the amount of the 24S component increases with each cycle of centrifugation, suggesting formation of the component as molecules of Fraction I protein are brought into closer proximity in the pellet. Secondly, the sedimentation constant of the 24S component and Fraction I are in the ratio of $1/\sqrt{2}$. Studies on the sedimentation behavior of dimers (44), trimers, etc., of large molecules have demonstrated that the sedimentation constant of the monomer to the dimer is in the ratio of $1/\sqrt{2}$; for the monomer and trimer, $1/\sqrt{3}$, etc. In passing, it may be mentioned that in one unusual preparation of cytoplasm, peaks which could be considered as dimers, trimers, and quadrimers of Fraction I protein were observed in the ultracentrifuge patterns.

Fraction I protein preparations from numerous plant species have been prepared by the ultracentrifuge method (18), and the products are in a high state of purity as judged by their behavior in the analytical centrifuge

and in the electrophoresis apparatus. The yield of Fraction I protein of 95 per cent purity amounts to about 5 mg. per gm. fresh weight of tobacco leaves.

Pirie (39) has prepared a protein from tobacco leaves, which in view of its properties to be described below, bears considerable resemblance to Fraction I protein. His method involves macerating the leaves in water, removing coarse fragments by low speed centrifuging, and then spinning the juice at high speed. The sediment which is deposited is extracted with water to yield the protein, while the particulate matter is left behind as an aggregated residue which does not dissolve.

Properties of Fraction I protein.—Experiments on the behavior and properties of Fraction I proteins have been confined to the work of two laboratories. Pirie (39) has isolated a protein from tobacco leaves closely similar in some properties to Fraction I protein prepared from spinach leaves (60) and other plants (8, 59). The proteins are seen to be unstable nucleoproteins in which the nucleic acid dissociates from the protein upon standing. The proteins have associated phosphatase activity and are capable of hydrolysing β -glycerophosphate and adenosine triphosphate among other phosphorus-containing substrates. A P-containing moiety can be removed from the protein and concentrated to where it contains about 8 per cent total P. The P is released from the protein, or the P-containing moiety, in two stages; 50 per cent of the total P is released rapidly in hot 1 N acid, but the remaining 50 per cent is only released upon total digestion with concentrated acid.

Eggman *et al.* (18) have extensively investigated the isolation and properties of Fraction I protein from tobacco leaves. Their preparation was isolated by centrifugation and was judged to be 95 per cent Fraction I protein by examination in the analytical ultracentrifuge. In contrast to Pirie's finding that his protein migrated as two distinct boundaries in the electrophoresis apparatus, the most mobile component making up 70 per cent of the total protein, this preparation migrates as a single, sharp boundary of great symmetry.

The yield of protein by Pirie is about the same as that found by Eggman *et al.* The nitrogen content of the protein lies between 13 to 15 per cent, in accord with Pirie, but the P content is generally less and the amount contained on the protein is much more variable than that observed by Pirie. Whereas Pirie's preparations contain from 2 to 3 per cent total P, Eggman's have ranged from 0.3 to 1.0 per cent total P which precipitates with the protein in the presence of trichloroacetic acid.

The most striking difference in the two protein preparations is found in their sedimentation behavior. Pirie's is completely removed from solution in 30 min. when spun at a force of about 80,000 g., whereas Eggman finds no protein deposited at this force. Rather, to remove as a pellet about 50 per cent of the Fraction I protein present in a neutral mixture of cytoplasmic proteins, a force of more than 100,000 g. must be applied for several hours. Evidently Pirie's protein preparation is of greater mass and more easily

sedimented. We would suggest that Pirie's protein preparation has been aggregated during preparation, particularly since in the absence of buffer the protein must have encountered acid conditions. In our experience, if a clear solution of cytoplasmic proteins is adjusted from pH 7.0 to pH 6.5, the solution becomes slightly opalescent within a few hours at 0°. With the appearance of opalescence some of the protein can now be deposited by centrifuging at a force of about 20,000 g., whereas the neutral protein solution will withstand a much higher centrifugal force without sedimenting. The speculation may be ventured that aggregation of Fraction I protein involves the "fixation" of nucleic acid to the protein in a less dissociable form. Such an interpretation might help to explain the reason for Pirie's preparation containing a greater and more constant amount of P than Eggman's.

In addition to P, Fraction I protein contains pentose, purines, and pyrimidines (39, 18). Adenine, guanine, cytosine, and uracil have been identified among the hydrolysis products of the protein, and it is estimated that the protein contains about 5 per cent nucleic acid (18). Pirie has obtained evidence for an enzyme in plant juice which accelerates the cleavage of the nucleic acid from the protein. Azide appears to inhibit the activity of the enzyme.

Physiological behavior and metabolic significance of the cytoplasmic proteins.—Most studies on the protein metabolism of plant leaves have dealt with the behavior of the gross protein content compared to extractable, nonprotein nitrogen fractions under differing environmental conditions. Information so derived is of little value in attempting to ascertain the metabolic relationships of various proteins, the sites and mode of protein synthesis, and the relationship of various specific proteins to the general metabolic pool. There is some evidence available, however, on the quantity of particulate proteins compared to the cytoplasmic proteins as affected by changes in external environment. Hanson (24) demonstrated a marked lowering of chloroplastic protein compared to cytoplasmic protein in leaves kept under conditions of drought. Detachment of grass leaves has been found by Wood *et al.* (64) to result in a change in the ratio of chloroplastic to cytoplasmic protein in Sudan grass, but in Kikuyu grass both types of protein declined at the same rate. Camus *et al.* (10) have found the ratio of chloroplastic material to cytoplasmic proteins of tobacco leaves to be markedly affected by conditions of controlled environment. It appears that a reciprocal relationship exists, such that increase in chloroplastic matter results in a lowering of the cytoplasmic proteins and vice versa. That the breakdown of proteins in detached leaves does not lack specificity is indicated by the finding that during a period when a 50 per cent reduction in total protein had occurred, the content of several enzymes remained unchanged [Axelrod & Jagendorf (4)].

Infection of tobacco leaves with tobacco mosaic virus has been observed to lead to a marked lowering in the normal protein content [Martin *et al.* (31); Bawden & Kassanis (5); Rischkov (43)], and closer analysis has shown this decrease to occur primarily in the cytoplasmic protein fraction (61).

It seems evident that one of the problems for the immediate future must include analysis of the physiological behavior of specific proteins. Some aspects can be attacked with presently available methods for resolving the cytoplasmic proteins, but others must await further advances. The successful application of isotope methods in this type of research will largely depend upon the efficiency of extraction and separation of proteins and, when accomplished, will undoubtedly lead to a far deeper insight into protein metabolism.

Finally, there remains the problem of the function of the cytoplasmic proteins in cellular metabolism. Fraction II proteins are known to display a variety of enzymatic activities (57), in contrast to Fraction I protein which is, at best, an uncertain phosphatase. The function of Fraction I protein remains unknown, but it is a reasonable expectation that a nucleoprotein present to such a large extent in so many leaves should have a major role in life processes. Is it an enzyme, a structural protein, or a "protein pool" utilized as a precursor of other proteins? While such speculative questions have no answers now, they may provide the impetus for further research in this important area of plant metabolism.

LITERATURE CITED

1. Alberty, R. A., *J. Chem. Education*, **25**, 426 (1948)
2. Anson, M. L., *Science*, **93**, 186 (1941)
3. Aronoff, S., *Botan. Rev.*, **16**, 525 (1950)
4. Axelrod, B., and Jagendorf, A. T., *Plant Physiol.*, **26**, 406 (1951)
5. Bawden, F. C., and Kassanis, B., *Ann. Applied Biol.*, **37**, 215 (1950)
6. Bonner, J., *Fortschr. chem. organ. Naturstoffe*, **6**, 290 (1950)
7. Bot, G. M., *Chronica Botan.*, **7**, 66 (1942)
8. Campbell, J. M. (Doctoral thesis, University of California, Los Angeles, Calif., 1951)
9. Camus, G. C. (Personal communication, 1951)
10. Camus, G. C., Eggman, L., and Wildman, S. G. (Unpublished data)
11. Chibnall, A. C., *Protein Metabolism in the Plant* (Yale University Press, New Haven, Conn., 306 pp., 1939)
12. Claude, A., *Advances in Enzymol.*, **5**, 423 (1949)
13. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., *J. Am. Chem. Soc.*, **63**, 459 (1946)
14. Comar, C. L., *Botan. Gaz.*, **104**, 122 (1942)
15. Crook, E. M., *Biochem. J.*, **40**, 197 (1946)
16. DuBuy, H. G., and Woods, M. W., *Phytopathology*, **33**, 766 (1943)
17. DuBuy, H. G., Woods, M. W., and Lackey, M. D., *Science*, **111**, 572 (1950)
18. Eggman, L., Singer, S. J., and Wildman, S. G. (Unpublished data)
19. Frampton, V. L., and Takahashi, W. N., *Phytopathology*, **36**, 129 (1946)
20. Galston, A. W., *Am. J. Botany*, **30**, 331 (1943)
21. Granick, S., *Am. J. Botany*, **25**, 558 (1938)
22. Granick, S., *Am. J. Botany*, **25**, 561 (1938)
23. Gray, G. W., *Sci. American*, **184**, 42 (1951)
- 23a. Gray, G. W., *Sci. American*, **185**, 45 (1951)
24. Hanson, E. A., *Australian J. Exptl. Biol. Med. Sci.*, **19**, 157 (1941)

25. Hanson, E. A., Barrien, B. S., and Wood, J. G., *Australian J. Exptl. Biol. Med. Sci.*, **19**, 231 (1941)
26. Hill, R., and Scarisbrick, R., *New Phytologist*, **50**, 98 (1951)
27. Hogeboom, G. H., *Federation Proc.*, **10**, 640 (1951)
28. Kabat, E. A., and Mayer, M. M., *Experimental Immunochimistry*, Chap. 26, 361 (Charles C Thomas, Publisher, Springfield, Ill., 1948)
29. Laties, G. G., *Arch. Biochem.*, **27**, 404 (1950)
30. Lugg, J. W. H., *Advance in Protein Chem.*, **5**, 230 (1949)
31. Martin, L. F., Balls, A. K., and McKinney, H. H., *Science*, **87**, 329 (1938)
32. McCalla, A. G., *Ann. Rev. Biochem.*, **18**, 615 (1949)
33. McKee, H. S., *New Phytologist*, **48**, 1 (1949)
34. Meneghini, M., and Delwiche, C. C., *J. Biol. Chem.*, **189**, 177 (1951)
35. Menke, W., *Z. Botan.*, **32**, 273 (1938)
36. Milner, H. W., Koenig, M. L. G., and Lawrence, N. S., *Arch. Biochem.*, **28**, 185 (1950)
37. Neish, A. C., *Biochem. J.*, **33**, 293 (1939)
38. Newcomer, E. H., *Botan. Rev.*, **17**, 53 (1951)
39. Pirie, N. W., *Biochem. J.*, **47**, 614 (1950)
40. Potter, V. R., Recknagel, R. O., and Hurlbert, R. B., *Federation Proc.*, **10**, 646 (1951)
41. Price, W. C., and Wyckoff, R. W. G., *Phytopathology*, **29**, 83 (1939)
42. Rabinowitch, E. I., *Photosynthesis and Related Processes*, **1**, Chap. 14, 355 (Interscience Publishers, Inc., New York, N. Y., 599 pp., 1945)
43. Rischkov, V. L., *Phytopathology*, **33**, 950 (1943)
44. Svedberg, T., and Pedersen, K. O., *The Ultracentrifuge* (Clarendon Press, Oxford, England, 1940)
45. Singer, S. J., Eggman, L., Campbell, J., and Wildman, S., *J. Biol. Chem.* (In press)
46. Smith, E. L., *Science*, **88**, 170 (1938)
47. Smith, E. L., *J. Gen. Physiol.*, **24**, 565 (1941)
48. Smith, E. L., *J. Gen. Physiol.*, **24**, 583 (1941)
49. Smith, E. L., and Pickels, E., *Proc. Natl. Acad. Sci.*, **26**, 272 (1940)
50. Smith, E. L., and Pickels, E., *J. Gen. Physiol.*, **24**, 753 (1941)
51. Wyckoff, R. W. G., Biscoe, J., and Stanley, W. M., *J. Biol. Chem.*, **117**, 57 (1937)
52. Steward, F. C., and Thompson, J. F., *Ann. Rev. Plant. Physiol.*, **1**, 233 (1950)
53. Stoll, A., and Wiedemann, E., *Fortschr. chem. organ. Naturstoffe*, **1**, 159 (1938)
54. Stoll, A., Wiedemann, E., and Rügger, A., *Verhandl. schweiz. naturforsch. Ges.*, **125** (1941)
55. Takahashi, W. N., *Phytopathology*, **31**, 1117 (1941)
56. Vickery, H. B., *Physiol. Revs.*, **25**, 347 (1945)
57. Wildman, S. G., and Bonner, J., *Arch. Biochem.*, **14**, 381 (1947)
58. Wildman, S. G., and Bonner, J., *Sci. Monthly*, **70**, 347 (1950)
59. Wildman, S. G., Campbell, J., and Bonner, J., *J. Biol. Chem.*, **180**, 273 (1949)
60. Wildman, S. G., Campbell, J., and Bonner, J., *Arch. Biochem.*, **24**, 9 (1949)
61. Wildman, S. G., Cheo, C. C., and Bonner, J., *J. Biol. Chem.*, **180**, 985 (1949)
62. Wildman, S. G., and Gordon, S., *Proc. Natl. Acad. Sci.*, **28**, 217 (1942)
63. Wood, J. G., *Ann. Rev. Biochem.*, **14**, 665 (1945)
64. Wood, J. G., Cruickshank, D. H., and Kuchel, R. H., *Australian J. Exptl. Biol. Med. Sci.*, **21**, 37 (1943)
65. Woods, M. W., and DuBuy, H. G., *Phytopathology*, **31**, 978 (1941)

PHYSIOLOGICAL ASPECTS OF LOW TEMPERATURE PRESERVATION OF PLANT PRODUCTS¹

By M. A. JOSLYN

*University of California, College of Agriculture,
Berkeley, California*

AND

H. C. DIEHL

*The Refrigeration Research Foundation,
Colorado Springs, Colorado*

INTRODUCTION

The purpose of this review is to evaluate the present status of research on the factors influencing the adaptability of fruits and vegetables to preservation freezing and the nature of the physiological changes that occur during preparation for freezing, freezing storage, and subsequent thawing. The industrial application of existing information is already well presented in the books by Tressler & Evers (1), Gortner, Erdman & Masterman (2), and Brown, Kunkle & Winter (3); in several textbooks on food technology, Cruess (4), Jacobs (5), Prescott & Proctor (6), von Loebecke (7); in the Refrigerating Data Book of the American Society of Refrigerating Engineers (8); in the bulletins of the U. S. Department of Agriculture, particularly those by Diehl *et al.* (9, 10); and of several of the state agricultural experiment stations, particularly those of Joslyn & Hohl (11), Wiegand (12), Woodroof (13), and Carlton (14). An extensive literature on home and locker freezing of fruits and vegetables exists, among which the publications of Tressler, Evers & Long (15), Tressler & Du Bois (16), Bedford *et al.* (17), Carlton (18), Diehl & Warner (19), Mrak (20), Plagge & Lowe (21), Winter & Hustrulid (22, 23) are typical. Dawson, Gilpin & Reynolds (24) have prepared a critical review of methods recommended for the preservation of foods by freezing and storage in home freezers and locker plants, assembled to promote uniformity in recommended procedures and in directing further research into those areas where available information is incomplete or conflicting. The available literature on various aspects of freezing preservation of foods has been surveyed by the Western Regional Research Laboratory of the U. S. Department of Agriculture (25), by Erdman (26), by Weil & Sterne (27), and by Anderson & Weil (28).

A physiological approach to freezing preservation was first presented in an early review by Diehl (29). The possible application of the early investigations on the freezing injury to plants and plant parts was inferred by Joslyn (30). A review of our present knowledge of enzyme activity in frozen

¹ The survey of the literature pertaining to this review was concluded in October, 1951.

vegetable tissue was made recently by Joslyn (31); Joslyn & Ponting (32) have published a review of enzyme-catalyzed, oxidative discoloration of fruit products; and the postharvest physiology and biochemistry of fruit has been reviewed by Biale (33).

The preservation of plant products by freezing depends on the retardation in rate or inhibition of the postharvest physiological changes, both enzymic and nonenzymic in nature, and in the control of microbial deterioration by decrease in storage temperatures. To preserve plant products for the relatively long storage period commercially desired, however, storage at temperatures well below those at which ice formation occurs is required. Biochemical changes are not sufficiently retarded or inhibited at temperatures just above or just below the freezing point of the product to allow storage with the minimum change in material for relatively long periods, even though Diehl (29) speculated that such temperatures might be used effectively and economically.

In order to accomplish the desired objectives of commercial preservation by freezing, temperatures below the freezing point of the product, usually 0°F. (-17.8°C.), or lower, are required. The combination of low temperatures and ice formation prolong storage life, but the latter also results in modification of the protoplasm which may terminate in phenomena of practical significance such as decided changes in product texture, color, and flavor. Diehl and his associates (unpublished data from investigations made during the years 1930 to 1935) observed that for many plant tissues there appears to be a critical temperature or temperature range between 0° and 10°F., especially between 5° and 10°F. When the tissues of halved apricots, for instance, were directly exposed to air temperatures ranging from -90°F. to +20°F., histological examination indicated that the cell contents presented a very different appearance at 10°F. from that at 0°F. or lower, down to -90°F. There was much more marked disintegration of the cell contents at 10°F. than at 0°F. The reason for this change is not known, nor is the relative importance of the liquid environment during freezing or during thawing understood. The phenomenon deserves closer investigation in the tissues of different types of plant forms. Berry and his colleagues, working in the same laboratory, also observed a marked difference in the killing of bacteria exposed to 0°F. and 10°F. A similar bacterial reaction was also observed by Bedford (34).

The undesirable effects that are produced by freezing, particularly on texture, are largely the result of this separation of water from the tissues as ice. In plant products this ice formation is largely irreversible. That is, in the over-all reaction of



the resorption of water upon thawing of the frozen, dehydrated gel system occurs to but a small extent. If it were possible to reach temperatures low enough to preserve the product for the desired period without causing crys-

tallization of water, low-temperature preservation might be greatly improved. Luyet, whose pioneering studies on the effect of crystallization and vitrification of water in tissues of simple life forms [Luyet & Gehenio (35)] are important, in personal discussions with one of the authors (Diehl), indicated that the practicability of such procedures in food preservation has yet to be demonstrated. At the present time we are limited to the selection of varieties or types of fruits and vegetables which are more tolerant to freezing, to harvesting them at optimum maturity for preservation, and to the development of processing methods which minimize the undesirable effects of ice formation. Preservation freezing is applicable only to those leafy, stem, and root vegetables which are cooked in preparation for use, because the irreversible, structural changes produced by freezing are of the same type as those produced by heating. Among these processing procedures, one that has not been generally applied to vegetables, although it has been used with some fruits, is the use of liquid environment about the product to be frozen. With vegetables, water or preferably a weak salt brine, has been used experimentally only; with fruits, sugar sirups have been used experimentally and commercially. Diehl and his associates at the U. S. Frozen Pack Laboratory in Seattle (unpublished data from investigations made during the years 1929 to 1935) repeatedly observed, in wet and dry packed vegetables, better retention of texture (turgor), color, and other desirable characteristics in the wet packed. Joslyn & Cruess (36) and Joslyn & Marsh (37) also observed these effects in their early investigations in this field. The mechanism of this protective effect is not known and the investigations were not pursued further at the time because the industrial trend was then, and has since remained, to dry frozen vegetables in non-liquid-proof paper containers. This phenomenon, corroborated in the experimental experience of some frozen food packers also, deserves careful and searching reexamination, especially since the freezing of concentrated fruit juices in hermetically sealed containers has now demonstrated the industrial feasibility of the use of such containers.

This review will be limited to the common physiological factors—varietal, maturity, etc.—which are involved in preservation freezing. Histological, physiological, enzymic, and nonenzymic changes occurring during freezing, freezing storage, and subsequent thawing which affect color, flavor, and texture will be discussed.

ICE FORMATION AND ITS EFFECT ON STRUCTURE

Much consideration has been given to the physical effects of ice formation in the studies of the low-temperature behavior of horticultural products. The rate of ice formation, particularly as affected by ambient temperature, and the size and distribution of ice crystals within the tissue, have been singled out for particularly intensive examination, possibly because of the marked popular emphasis on these phenomena during the early years of frozen foods development. The earlier, applied microscopical observations have

been reviewed by Diehl (29), who pointed out that the belief that ice forms within the cells of plant tissues, which are then injured by the crushing and piercing action of the ice crystals, was not supported by the early physiological investigations in this field. The phenomenon of ice formation in plant tissues has been observed by physiologists for many years. Their investigations into the nature and function of many physiological and physico-chemical principles operative in plant-tissue freezing are applicable to an understanding of the limitations of preservation freezing to retention of the desirable color, flavor, and texture of plant products.

The death of plant tissue when the temperature is lowered below the freezing point, with the accompanying formation of ice crystals, results in wilting and pronounced texture and color changes. These changes in appearance of the plant or plant part and in the protoplasm itself have been described often in the early literature, perhaps first by Sachs (38) but particularly well by Müller-Thurgau (39) in his studies on the freezing to death of plant tissues, as well as by Molisch (40) and Matruchot & Molliard (41).

The early literature on the killing of plant tissue by low temperature was reviewed by Chandler (42), who also presented data supporting the view that killing from cold results from the withdrawal of water from the protoplasm to form ice in the intercellular spaces. The various theories proposed to explain low-temperature killing are discussed by Chandler (42, 43, 43a), Diehl (29), and Luyet & Gehenio (35). Chandler & Hildreth (44) pointed out that the data obtained by Maximow (45) on the freezing of red cabbage and *Tradescantia* leaves in various solutions did not support the view that death in frozen plant tissue was caused by either an injurious concentration of the sap or denaturation of the protoplasmic constituents by separation of protective substances. They showed that the response of pollen grains to freezing supported Maximow's view that the killing of protoplasm at low temperature was probably the result of a direct effect of the ice masses. Just what this effect is, however, was not established, although the authors suggest either a rupture of the protoplasm or a mechanical coagulation of the protoplasmic colloids.

In this connection, Finnegan (46) has demonstrated experimentally the development of pressures of considerable magnitude and possible physiological importance resulting from expansion within the tissues or masses of foods during freezing. Since tissue growth does not occur equally in all parts of a plant organism and the cell walls of tissues or the boundary zones between different types of tissues within such an organism are not all of the same character and thickness, it is possible that pressures such as Finnegan has demonstrated can effect the disruption of tissue areas. Hence, the disruption of cells and tissues by freezing in a living organism caused by growing ice masses can be the result of expansion pressures rather than piercing of cell walls by needle-like ice crystals, an extremely popular concept in early food freezing technology which, however, was based on very little experimental evidence. That the expansion pressure of ice masses occurring

within cells under certain freezing conditions may also have a part in this process need not be gainsaid.

It would seem scientifically important, however, that the investigator, especially the one concerned with the development of sound freezing technology, thoroughly acquaint himself with the scientific literature of freezing phenomena. The technology may then at least rest on reasonably scientific deduction, even where experimental data are meager or not available, rather than on plausible and popularly acceptable concepts that are not scientifically logical or demonstrable. That much research remains to be done in this field is manifest. This area is one of the most challenging and potentially productive for physiological and physico-chemical research.

Luyet *et al.* (35, 48 to 53), however, presented data to show that, if plant cells are killed by freezing, coagulation of the protoplasm does not occur simultaneously but usually some time after freezing and then is morphologically different from that which is produced by such other lethal agents as heat. Luyet & Condon (50), for example, found that potato tissue, when frozen under conditions in which only 35 per cent of its water congealed, was undamaged; but that when 40 to 70 per cent of its water congealed and the temperature dropped from -0.5 to -3.5°C. , the cells died. A "hard frozen" cell is comparatively unstable. Luyet & Gehenio (49) studied the complex behavior of living tissue on freezing and explain the occurrence of a double freezing point of such tissue by the assumption that water occurs in four states. These investigations indicate the complexity of the problems involved in the determination of the mechanism of freezing injury to plant protoplasm. Since fruits and vegetables are commercially stored much longer, and at temperatures much lower, than would be required to kill them, the data obtained in physiological studies of the low-temperature killing of plant tissues is not entirely applicable to the explanation of histological changes occurring in such tissues. However, much is to be gained by the investigator of freezing phenomena who is fully cognizant of the previously published extensive investigations in this field.

The earlier investigations of the effect of freezing on tissue structure, such as those carried out by Woodroof (55), were based on the measurement of loss of juice ("drip") associated with loss of structure on thawing and microscopic observation of free hand sections at room temperature. Since the drained weight of defrosted frozen plant tissue depends on the method of freezing, the conditions and length of freezing storage, the method of thawing, and the condition of drainage, the loss in weight or the volume of the fluid separated, is, at best, only a rough empirical measure of changes in structure. Microscopic examination of free hand sections or even sections made by the freezing microtome are also subject to considerable error and are difficult to interpret in terms of actual structural changes. Refinements have been introduced in both methods of histological examinations. The thawing of frozen samples under petroleum ether prior to separation of the liquid phase was introduced by Kaloyereas (56). Kethley *et al.* (57) devel-

oped a new test for gauging leakage from frozen fruits and berries. There are still differences of opinion as to the best procedure for determination of leakage of juices as controlled experimental studies of the several factors involved have not been made.

Woodroof (58, 59) improved his original microscopic procedure by sectioning the tissues with a sliding microtome while they were still frozen and then floating the frozen sections in a chilled killing and fixing fluid before examination at room temperature. MacArthur (60, 61) used a similar technique. Lee *et al.* (62, 63, 64) also prepared sections while the material was still frozen and prepared photomicrographs with and without thawing of the sections. Lebeaux (65) introduced the use of the petrographic microscope for the determination of ice crystal formation. Thin sections of frozen material were examined and analyzed by means of a polarizing microscope. While these investigations were of limited scope, they opened an entirely new approach to the investigation of the effects of ice formation on living tissues. It is to be hoped that further research in this direction will be undertaken.

Woodroof (58) found that for microscopic studies, fruits and vegetables suitable for freezing were conveniently divided into four groups: (a) growing stems—asparagus, rhubarb, broccoli, spinach—consisting largely of meristematic tissue; (b) mature fruits—strawberries, raspberries, dewberries, figs, cherries—consisting of very thin-walled parenchyma cells with large intercellular spaces; (c) immature seeds—peas, beans, lima beans, corn—containing starch capable of losing or absorbing water; and (d) young leafy tissues—spinach, broccoli, green bean hulls—similar to young stems but with more surface exposed. Woodroof ascribed the loss of structure and flabbiness of frozen and thawed plant tissue to: (a) puncturing of cell walls by ice crystals; (b) withdrawal of more water into intercellular spaces during freezing than is reabsorbed on thawing; and (c) destruction of the colloidal complex of cells. Weier & Stocking (66), criticized his observations and interpretations on the basis of cell structure and cell physiology. They reviewed the meager data available on histological changes brought about by freezing and briefly summarized some of the factors involved in freezing. Hohl (67) briefly discussed the practical applications of histological research to food processing and stressed the anomalous behavior of apricot skins on freezing. Although subjectively a toughening of the skins of apricots occurs during freezing, yet objectively this phenomenon could not be demonstrated.

The literature dealing with histological changes brought about by freezing is not extensive and is neither detailed enough nor sufficiently critical to establish the type and actual extent of the various changes likely to occur in the cell walls, cell contents, and middle lamella. Even in the interpretation of the over-all effects of freezing conditions (temperature, rate of heat transfer, etc.) there is marked disagreement. Woodroof (58), for example, concluded that rate of freezing markedly affected the size of ice crystals,

both inter- and extra-cellularly, and consequently the resulting damage to the plant cells. MacArthur (60, 61) agreed with him that slow freezing resulted in more tissue destruction by large, internal ice crystals than more rapid freezing, particularly in asparagus. Lee *et al.* (62) found that although slow freezing resulted in the formation of large veins of ice in peas and snap beans, no injury was evident when the product was thawed and no significant differences in palatability could be detected between products frozen slowly and those frozen rapidly. Similarly, the rate of freezing had no effect on the palatability of spinach, corn, and lima beans although with asparagus, differences in texture were found with different rates of freezing (63). This result was noted in the preliminary observations made by Joslyn & Marsh (68). Lee *et al.* (64) reported no difference in texture, microscopic appearance, or palatability in strawberries, raspberries, and sliced peaches packed in sirup whether frozen very slowly, very rapidly, or at intermediate rates. Lee & Johannesen (69) recently reported that differences in texture as measured by crushing resistance of cooked asparagus spears were unaffected by rate of freezing. Lebeaux's technique (65) has not yet been applied widely enough to test and establish fully the validity of the scientific deductions indicated by his published results. Further critical investigations by competent plant and animal cytologists are necessary to evaluate the histological changes produced by freezing and freezing storage and the physiological and biochemical factors involved. The mechanism of freezing in food products can be studied by investigating the behavior of model systems as well as by histological techniques. Cook & Lusena (70) employing the former method recently found that continuous films, such as "Cellophane," showed a high degree of resistance to penetration by ice crystals and inferred that undercooling of cell contents is the mechanism predominantly responsible for the uniform distribution of ice crystals within and between cells. A very interesting and fertile field of research which will demand vision, time, and patience is awaiting the investigator interested in this relatively unknown area of freezing behavior.

COLLOIDAL ASPECTS OF PRESERVATION FREEZING

Few serious contributions have been made to our knowledge of the response to freezing of colloids such as occur in plant cells, although it was early reported that changes in colloidal systems involved are one of the factors limiting texture retention. Some aspects of the behavior of colloids to freezing and thawing were investigated by Luyet and his co-workers (35, 48 to 54) in their development of the theory of the vitreous state of frozen protoplasm. Nord and his associates (71 to 78) and others have made significant contributions to the investigations of the properties of naturally occurring organic and inorganic colloids at low temperatures. The conditions under which inorganic colloids undergo aggregation and disaggregation and the factors which influence this change in particle size (temperature, time of cooling, rate of cooling, concentration of disperse phase, nature of the sol-

vent) have been fairly well established, but the behavior of organic colloids, and particularly those occurring naturally, is still largely unknown. All that has been established is that on freezing, either aggregation or disaggregation of particles can occur, depending largely on concentration, but a complete survey of all the factors involved has yet to be made, as pointed out by Leichter (78).

It is interesting to note that Gorke (79) first pointed out that proteins can be precipitated by freezing, when the concentrations of salts increase as a result of the removal of water from the cell sap of plant tissues. While his concern, as that of Nord (73), was with the death of living cells by freezing, it is not illogical to give some attention to these hypotheses as they may apply to turgor and texture changes in the tissues of frozen and subsequently defrosted plant and animal tissues in connection with freezing preservation.

Changes in protoplasmic structure on freezing are believed to be influenced by both external factors (extent of ice formation, duration, and rate of ice formation) and inherent characteristics such as water content, colloidal nature, and age. Harvey (80) early found that the changes in pH in tissues subjected to desiccation, osmosis, or freezing were of the same order of magnitude. Pickett (81) found that the juice expressed from frozen fruits was appreciably lower in pH value and higher in total titratable acidity than that of unfrozen fruit. Holzapfel & Nord (82), using model colloidal systems, showed that decreases and increases in pH after freezing depend on the degree of dispersion and type of gases adsorbed. These changes of pH in colloidal systems are but one of the methods of following the behavior of colloids on freezing. An even more important factor is the original concentration of water in the tissues and its distribution between "free" water (capable of being changed to ice) and "bound" water (which does not freeze at temperatures employed in freezing practices). These factors are discussed in some detail by Luyet & Gehenio (35). Daughters & Glenn (83) determined the total and bound water content of asparagus, corn, beans, raspberries, rhubarb, spinach, and strawberries but show no data that variations in bound water content were actually related to differences in response to freezing. On the other hand, practical experience has indicated that plant tissues lower in water content and higher in soluble solids content, for example, ripe figs, respond to freezing with a minimum of change in texture. This response was also noted in partially dehydrated fruit and vegetable products (84, 85).

Friess (86) reported the results of mixing small amounts of plant tissue extracts with cupric chloride solution and allowing crystallization of the solution to occur under definite conditions. He obtained crystalline patterns observed microscopically to vary with the species of tissue and with the treatment given the tissue (heating, freezing, drying, or chemical). Although differences observed with treatment were quite marked, the interpretation of these changes in terms of variations in colloidal characteristics was not attempted. It is not known whether this interpretation will be possible.

Maximow (45) some years ago found that the resistance of plant tissue to killing by freezing was markedly increased when it was exposed to low temperatures either in its own sap or in sugar solutions. This effect was originally proposed by Diehl (29) and by Joslyn (30) as an explanation of the well-established improvement in appearance and palatability of fruit frozen in sirup (11). Maximow, however, found that the protective effect of the solutions which he used was related to their eutectic properties and not to their osmotic properties. The improvement in color and flavor retention of cut fruit and berries frozen in sirup, however, is not related to the osmotic or eutectic properties of the sirups. The available data on the mechanism of the protective effect of sirups is discussed by Joslyn & Hohl (11) and by Joslyn (87).

The absorption of sugar by fruit frozen with added sugar or sirup has long been considered desirable to improve preservation of color and flavor and to yield a better tasting product. The undesirable effects of undissolved dry sugar are well known in the industry and precautions are now taken to obtain uniform mixing of fruit and dry sugar. In the evaluation of the most desirable types of sugar or sugar sirups to be used in the preparation of fruit for freezing storage, and of the best methods of using these sirups, it is necessary to know the ease with which they spread over fruit tissues, are held by them, and are absorbed by the fruit. In canned fruits, exchange of soluble solids between fruit and sirup occurs, and shortly after canning an equilibrium is reached. The factors influencing this exchange have been fairly well established for canned fruit, but not for frozen fruit. Delayed freezing, impregnation-de-aeration, and other methods have been tried to improve this exchange. The effects of replacing sucrose by other sweeteners have been investigated (88, 89), and investigations have been conducted on the prevention of sucrose hydrate formation (90, 91), but more basic information in this field is needed.

The addition of low methoxyl pectinic acids to the sirup in which berries were frozen was found to produce an improvement in texture [Buck *et al.* (92) and Wegener *et al.* (93)], but this result was not obtained with other fruits. Lee *et al.* (94) found that pectin tends to reduce browning of sliced apples, but the nature of this effect was not established.

ENZYME-CATALYZED CHANGES IN COLOR AND FLAVOR

Joslyn (31, 95) summarized and evaluated the available data on enzyme activity in frozen vegetable tissue responsible for changes in flavor and color occurring during freezing storage. Although the general nature of such changes is known, it has not yet been established just what enzyme systems and substrates are involved. The changes in flavor occurring in frozen, raw vegetables have been ascribed to autolysis, proteolysis (96, 97), glycolysis, and more recently to oxidative rancidity of lipids (98). Although the nature of the products formed during freezing storage of unsalted vegetables is not known, the investigations carried out by Joslyn and his associates dur-

ing the period of 1935-1940 (31) indicate that aldehyde accumulation occurs in artichoke hearts, asparagus, Brussels sprouts, green snap beans, lima beans, and squash. In broccoli and probably spinach there was an indication of accumulation of alcohol. Although acetaldehyde is not the cause of the off-flavor, it appears to be the one of the by-products of the reaction producing the unidentified compound or compounds which give the product the off-flavor. Gutterman *et al.* (99) clearly demonstrated that whenever the acetaldehyde level in frozen peas and asparagus exceeds a certain point, organoleptic taste panels have invariably classed the product as being of inferior quality or inedible.

It was early established (36, 100 to 105) that production of off-flavors in frozen vegetables could be inhibited by scalding them in steam or hot water prior to freezing and freezer storage. Such scalding, when properly carried out, resulted not only in good flavor retention but also in improved color retention. Empirical observations indicated that catalase activity or peroxidase activity could be used as indices of the extent of heat inactivation of the enzymes responsible for the development of off-flavors. There is still disagreement among the investigators in this field as to which of these enzymes more closely parallels the rate of heat inactivation of the enzyme systems actually involved in off-flavor production. Joslyn and co-workers (31) have found that peroxidase activity, when determined by the use of an appropriate substrate (guaiacol), more closely parallels off-flavor production. Lindquist and associates (106) reported recently that for Brussels sprouts and peas, at least, this conclusion is true. Campbell (107), however, still prefers the catalase test for peas. Keirmeier (108) in critically evaluating the use of lability of enzymes as a guide to the technical treatment of raw material, pointed out that the determination of enzyme activity after different treatments constitutes a valuable criterion only if the experimental conditions, such as rate of freezing, duration of super cooling, etc., are constant and reproducible. He stressed that the action of enzymes in frozen foods depends not only on the original enzyme content of the plant tissue, but also on the effect of freezing which may fundamentally alter the form in which the enzyme complex exists (lyo-, desmo-, or endo-enzymes). The basis of the use of enzyme activity as an index of adequacy of scalding treatment was discussed in some detail by Joslyn (31).

The available information on enzyme-catalyzed, oxidative browning of fruit products was summarized and evaluated by Joslyn & Ponting (32). While it is fairly certain that polyphenol oxidase is the principal enzyme involved, and that discoloration will not occur until the naturally occurring ascorbic acid is oxidized, the nature of the phenolic substrates in fruits which undergo enzymic oxidation and polymerization to dark colored pigments is still unknown. Even the mechanism of pigment formation from simpler phenolic substrates is not known in detail. Melanin formation by the tyrosinase-induced oxidation of tyrosine, although widely studied, is so far known only in general outline. The biochemistry of melanin formation has

been reviewed most recently by Lerner and Fitzpatrick (109), who point out the common characteristics of the tyrosinase from plants, lower animals, and humans, and discuss the mechanism of tyrosine oxidation.

Guadagni and co-workers (110) measured the total phenolic compound content ("tannin" content) of over 50 varieties and hybrids of peaches, their phenolase enzyme activity, and the rate of tannin oxidation in the ground peach tissue. Only a fraction of the total phenolic substance content was found to be oxidizable under their experimental conditions, but the susceptibility of the different varieties of peaches to enzymatic browning was directly related to the amounts of oxidizable tannins present. Varieties whose oxidizable tannin value was in the range of 18 to 19 mg. per 100 gm. exhibited little or no browning on prolonged exposure to air regardless of polyphenolase activity. This limitation in tendency to browning with "tannin" content was reported previously by Kertesz (111) for the Sunbeam variety.

The nature of the so-called "tannins" of fruits, which have been implicated as chromogens, has been investigated critically by Johnson and associates (112, 113), who succeeded in the isolation of the tannins of peaches and showed that the characteristic ultraviolet absorption spectrum of polyphenolic substances from various fruits could be used in their investigations. Johnson *et al.* (113), using a synthetic anion exchange resin for the isolation, and paper chromatography and ultraviolet absorption techniques for characterization and identification, isolated among the phenolic substances, *dcatechin*, chlorogenic acid, and the 3-monoglucoside of cyanidin. Clark & Levy (114) introduced chromatographic separation of tannins from salt solutions and newer methods for the separation and investigation of the naturally occurring phenolic substances are under investigation at Cambridge [Bate-Smith & Westall (115)] and elsewhere. With extension of investigations of this type, progress can be made in our knowledge of enzymic browning and the factors controlling it. At present, control of enzymic browning in frozen fruits is carried out by selection of varieties least susceptible to discoloration, removal of oxygen from the fruit tissue as well as from the surrounding atmosphere, reduction in pH, addition of anti-oxidants, and heat inactivation of phenolase. The most complete data on the thermal rate of inactivation of polyphenolases is that of Dimick *et al.* (116) for fruit purees.

VARIETAL AND MATURITY FACTORS

The importance of varietal adaptability of fruits and vegetables to preservation by freezing was recognized by the early investigators shortly after the demonstration of noticeable differences in response to freezing of the various commercially available types of produce. Systematic investigations of the varietal adaptability of peaches and strawberries were carried out by Caldwell and his collaborators at the Bureau of Plant Industry under Eastern conditions (117 to 120) and these were paralleled by similar but even more extensive and detailed studies carried out co-operatively with the State Agricultural Experiment Stations in California, Oregon, and Washington by

Diehl *et al.* (121). These co-operative investigations by the Bureau of Plant Industry and its field stations were extended to varieties of peas, snap beans, corn, and spinach in 1934. The data on comparative studies of varietal suitability for freezing preservation of peas, green or snap beans, lima beans, and sweet corn grown under Eastern conditions was summarized by Caldwell and his associates in 1940 (122). Extensive vegetable varietal trials to determine the cultural behavior and frozen-pack quality of vegetables and fruits grown in the Pacific Northwest region were started in 1936 by Diehl and his associates in co-operation with Schwartze at the Puyallup and Prosser Branch Stations of the Washington Agricultural Experiment Station, with Bouquet and Wiegand of the Oregon Agricultural Experiment Station, and with Waldo of the U. S. Bureau of Plant Industry at Corvallis, Oregon (123a, 123b, 124). These early investigations have been extended, in co-operation with several State Agricultural Experiment Stations, by the U. S. Bureau of Plant Industry, Soils and Agricultural Engineering, the U. S. Bureau of Agricultural and Industrial Chemistry, and by several of these State Stations independently. These early investigations were carried out for the purposes of (a) comparing the varieties already used commercially, (b) evaluating promising European and American stock, and (c) establishing the most desirable characteristics to serve as a basis for possible improvement of varieties by plant breeding methods. A considerable number of commercial seed companies co-operated in this undertaking.

The varietal characteristics desirable for certain important vegetables and fruits intended for freezing preservation established by these early investigations (9, 11, 12, 121, 122, 123a, 123b, 124) were widely studied and applied in the selection of varieties and types for commercial freezing preservation and by plant breeders in the development of new varieties. As an example, it was established that only the peas with green seed coats and green cotyledons were suited for freezing preservation and that the thickness of the periderm in corn was an important factor in obtaining frozen corn of desired quality.

Initial studies were made for two years on replicated row plots, but later small acreage plots (1/10 or 1/20 of an acre) were studied on the relatively few varieties which survived the rigorous tests for quality and important growth and yield characteristics. The larger acreage tests permitted evaluation of yield characteristics and behavior under mechanical harvesting and field handling conditions. These factors are, in the final analysis, equal in importance to the desired quality characteristics of the variety under processing, freezing, and freezer storage conditions. The history of these many varietal experimental samples of plant or tree selection was known from seed to the moment of test consumption.

The selection of the ideal varieties of fruits or vegetables, or their development by plant breeders through the establishment of desirable quality characteristics which can be genetically reproduced, is one of the most important phases of freezing preservation research. Much more knowledge is

needed in this field. More information is necessary to establish the effect of harvesting and handling practices, to define the maturity and quality of the raw material, and to better define response to freezing, thawing, and preparation for use. The desirable variety must be capable of yielding products of high quality in the raw state; must suffer the minimum change in color, flavor, and texture during freezer storage and preparation for table use; must yield well under the given growing conditions; and must be adapted to mechanical harvesting and mechanized handling during preparation for processing.

The oxidative browning already mentioned is a major problem of successful freezing preservation in the case of light-colored fruits such as apricots and peaches. The discovery of ideal peach varieties without browning tendencies and with desirable quality characteristics, especially of flavor, color, and texture, would be of great economic significance to the frozen foods industry and save much effort and money in processing procedures and equipment (heat blanching, use of anti-oxidants, evacuation under sirup, and so forth). Many quality factors can be much more desirably obtained by plant breeding and varietal selection, although these admittedly require time as well as research and development effort, than by modifications of processing procedure designed to prevent or retard some undesired physiological phenomenon. The value of intimate collaboration between plant scientists and food technologists is nowhere better illustrated than in the freezing preservation of fruits and vegetables. A beginning only has been made in the last quarter of a century toward the accumulation of much needed knowledge in this field.

The evaluation of quality of frozen fruits and vegetables has received considerable attention of late. For the evaluation of those factors which cannot be measured objectively as yet, the careful selection of taste panels coupled with rigid statistical analysis of the results of taste differences has done much for more careful and critical taste appraisal. Methods for the objective testing of quality in vegetables have been developed and introduced by Kramer in Maryland (125 to 129), Lee in New York (130 to 133), Makower (134), and others (135). Quality evaluation now can be more carefully controlled and carried out. See, for example, Gould *et al.* (136, 137) and Robinson *et al.* (138). The evaluation of the effect of handling practices on quality in peas has been studied by Campbell & Diehl (139) and the role of bruising and delay in handling on the development of off-flavor in peas by Makower & Ward (140). Boggs *et al.* (141) previously had investigated the peculiar toughening of the skins of peas resulting from mechanical injury.

The important effect of maturity on quality has been investigated extensively for peas by Nielsen *et al.* (142). Objective methods of measuring maturity of asparagus, lima beans, corn, and peas have been developed by Lee *et al.* (130-133). Although the tenderometer and similar texture meters under properly controlled conditions are suitable for field tests, more ex-

tensive information on the histological and biochemical changes occurring during growth and maturation of vegetables are necessary as a basis for the development of a useful index of maturity by field men.

As a result of recent investigations in this field, the stage of maturity and the variety most adaptable to freezing preservation can be more closely defined for some of the more important commercial vegetables, but a more careful and basically sound evaluation of varietal quality and maturity for berries and fruits still must be developed. The inherent varietal characters are still to be completely appraised and evaluated. The importance attached to these factors can best be summarized in the words of Heiss (143)—“Conditions of growth and choice of variety have a greater influence on the quality of frozen fruit and vegetables than technical factors.”

HEAT TRANSFER AND ENGINEERING FACTORS

Any discussion of the physiological aspects of freezing preservation must have reference also to the methods by which the heat transfer from the organism or product to the refrigerant is effected, insofar as the rates of heat transfer and the resulting character of the ice formation has a bearing on the subsequent behavior of the tissues of the organism or product. These freezing methods and equipment are discussed briefly in the comprehensive book by Tressler & Evers (1). This reference will suffice to acquaint the reader with the inventive and engineering developments employing refrigerated air, liquid, or contact surfaces as a practical means of bringing about heat transfer. We are not concerned with the variations of procedure and equipment arising from inventive genius or personal predilection or with the popular claims made for their effectiveness. We are concerned with the adequacy and efficiency of these procedures in effecting rates of heat transfer and types of ice formation which physiological study show to be significant in the behavior of the frozen organism.

Research on low temperature preservation of plant products, however fundamental, must be related to the possibilities of practical achievement of the desired rates of heat transfer. Other factors must be considered also. If moving air is to be used as the secondary refrigerant, then the desiccative effect of such air, especially upon unpackaged products, has a bearing on product behavior quite aside from the primary considerations of heat transfer from product to refrigerating medium.

Enthusiasts for individual freezing methods have made many claims for the effectiveness of particular procedures. Some of these claims have been speculative and hopeful, but not always unjustified. Some have been based on actual research and the data accumulated thereby. In some cases, research workers and enthusiasts have investigated entirely objectively some of the fundamental factors of these several freezing methods, as did Woodroof (58) and Finnegan (46, 144, 145).

The rate at which a commodity freezes is dependent upon two sets of factors, those concerned with the nature of the product—its thermal properties, dimensions, and initial temperature—and those concerned with the

freezing medium—its thermal properties, temperature, and relative motion. Investigation of the factors influencing rates of temperature change during freezing and subsequent thawing have been reported by Joslyn and Marsh (146, 147, 148), Finnegan (149), Nicholas (150), Phillips (151) Kethley *et al.* (57) and others (see ref. 1 and 2). Specific heat and heat conductivity data on fruit and vegetable products are quite meager. Short (152) and Kethley *et al.* (153) published the few experimentally obtained data on the specific heat and thermal conductivity of fruits and vegetables that are available. Woolrich (154) has summarized the available data on the latent heat of foods. Riedel of Karlsruhe, Germany, developed an improved apparatus and methods for thermal conductivity measurements (154a) and has studied extensively the thermal conductivity of organic liquids (154b), sugar solutions, fruit juices, and milk (154c, 154d) and has recently published charts on the quantity of heat that must be removed from juices, fruits, and vegetables in the course of chilling and freezing (154e).

All of the significant influences which the methods of bringing about heat transfer and freezing entail cannot be discussed here. As an example, however, the physiologist as well as the food technologist cannot overlook the fact that methods involving the envelopment of a foodstuff, either packaged or unpackaged, in a liquid secondary refrigerating medium bring about a relatively rapid rate of heat transfer (sometimes at comparatively moderate freezing temperatures) and so may have both physiological and economic significance. Methods involving liquid refrigerants and suitable containers have not been more rapidly adopted by the food freezing industry to date for a great many reasons—historical, engineering, and technological.

Furthermore, there exists an urgent need that the results of physiological research in this field be brought immediately to the attention of refrigeration engineers and inventors. Obviously, if the latter can be guided more directly and promptly to the physiological phenomena which freezing creates in foodstuffs, the development of engineering methods to bring about essential heat transfer and freezing sequences will be expedited. Much time, effort, and money can be saved by closer integration of the findings of physiologists and the action of inventors and engineers.

Finally, by the same integration, the physiologist will be, as he should be, more intimately aware of what is feasible in engineering development to implement the desired heat transfer and freezing requirements of food commodities; and the refrigeration engineer will understand, by the same token, more precisely what essential physiological objectives must be satisfied in order to create an effective and efficient method of food preservation by freezing. Much remains to be done in these fields of human endeavor and cooperation as well as in the uncovering of fundamental facts about the effects of ice formation on living tissues of plant and animal organism. Here lies an unusual challenge to both physiologist and inventor-engineer.

FREEZING STORAGE AND DISTRIBUTION FACTORS

Although the focus of lay and research attention in the low temperature

preservation of foods has been on the phenomena which characterize and accompany the change of state, primarily of water, in foodstuffs, scientific propriety demands that one emphasize also the importance of the physiological changes which occur in the products so preserved during their life subsequent to freezing. This consideration is especially true of fruits in which the enzymic activities are not customarily inactivated by heat as they are with vegetables.

This phase of freezing preservation has been, so far, very inadequately explored and yet it is probable that there is no food freezing investigator who is not aware by repeated observation that marked and significant changes may occur during the storage life of frozen foods. The causes and complete nature of these changes are presently understood only imperfectly. Expediency and lack of specific information have made it obviously necessary that many products be stored together at freezer temperatures which have been deemed, sometimes on meager scientific evidence, to be adequate. Actually, in the present state of industrial development they have been economically adequate, but continuing complacency on the part of scientific investigator, development engineer, and frozen food producer is not thereby indicated or to be encouraged.

Some investigators such as Diehl (29) and Woodroof (13) have suggested that each product may eventually have an optimum freezing and freezer storage temperature. Certainly, analogy in the field of food preservation by heat and drying suggests the logic of such an assumption, even if there were no experimental evidence presently to sustain it. Admittedly there are, as in so many phases of freezing preservation, only initial observations to sustain this logic, but these indicate that further research may uncover very significant facts which may eventually materially affect the industrial procedures.

Here again is a potentially fertile research field for the physiologist interested in the preservation of foods at low temperatures. The practical concern that it may not be economically feasible to provide freezing or freezer storage conditions for each individual frozen foodstuff need not concern us seriously at the moment. As is true in so many instances, we need the scientific facts before we decide upon the economic feasibility of an industrial procedure.

DESTRUCTION OF MICROORGANISMS AT LOW TEMPERATURES

Although a review of the extensive literature on the survival and destruction of microorganisms in frozen plant products is out of place here, certain aspects of the destructive effect of low temperatures have a bearing on our discussion of preservation freezing. Certain aspects of the microbiology of frozen foods have been reviewed by Bedford (34), Haines (155, 159 to 161), James (156), Wallace & Tanner (157), Berry & Magoon (158), Prescott & Tanner (162), and Pederson (163). It was pointed out in these reviews that numerous species, including even some of the pathogenic forms can grow at 0°C., and in some cases, considerably below that temperature [Berry &

Magoon (158) and Haines (164)]. An organism may fail to grow at subzero temperature because of ice formation in the medium rather than because of cold alone [Berry & Magoon (158); Berry (165)]. Haines (159 to 160) suggested that most bacteria cease to grow at -10°C . but at this temperature there could still be slow chemical changes by enzymes secreted by bacterial cells. The nature of the inhibiting effect of ice formation on growth is not known but may be similar to the effect of ice formation on enzyme activity [Joslyn (31)]. The destruction of microorganisms by low temperatures and ice formation has been investigated over a number of years, but although the general phenomena has been described there is as yet no completely adequate explanation of the effects observed. Haines (161) suggested that one factor in the destruction of bacterial cells by freezing is the flocculation or crystallization of their cellular proteins. Other explanations are discussed in the reviews mentioned above. Some aspects have been investigated in more detail recently by van Eseltine *et al.* (166) and Weiser *et al.* (167, 168), among others.

It is a well-demonstrated fact that bacteria may be more rapidly killed at a moderate freezing temperature than under hard freezing conditions. The phenomenon was noted by Prescott and co-workers (169) in studies in frozen foods in 1932. Shortly after, Berry (165), working with frozen pack fruits, reported kills of 99 per cent in 13 months' storage of blackberries at 15°F ., while the kill at 0°F . was only 40 per cent. Strawberries and raspberries under observation for shorter periods showed the same trend. Bedford (170) reported similar findings on fish. The facts at first glance seem illogical and still await full explanation.

Considering the small size of the bacterial cell and the well-known fact that liquid in a very thin film or in a capillary resists freezing [Bigelow & Rykenboer (171)], it is doubtful that ice is formed within the cell at -17°C ., or even below. Rather, the cell is desiccated, water being pulled out into the surrounding medium or the intercellular spaces, to build up ice. This process is only moderately lethal; given a large number of cells, some will stay alive for long periods. In fact, cultures of bacteria may be preserved by "lyophilization," i.e., hard freezing followed by sublimation of the ice. The chances of a cell's surviving appear to be fewer if desiccation is incomplete, as is the case at light freezing temperatures. In popular language, one could say that in one case the cell is hardened or cured, while in the other it remains intact and capable of functioning but cannot because of the cold. Chemical changes—denaturation of protein or what not—must take place more readily at moderate freezing temperatures, where ice formation is incomplete. These changes are irreversible and inconsistent with the life of the cell.

Weiser & Osterud (167) differentiate between the rapidly acting or "immediate" death caused by freezing and thawing per se, which occurs at a brief stage in the freezing process during which extracellular ice formation is being completed, and a "storage death," which is a direct function of time and temperature. Immediate death is marked but does not vary with the in-

tensity of freezing temperature. The rate of storage death at the higher freezing temperatures is very rapid and is much greater at temperatures above -30°C . than at or below -30° . Weiser & Hargess (168) also differentiated between the increase in mechanical pressure and concentration of materials in the intercrystalline film resulting from ice crystallization and vitrification phenomena. Vitro melting at -195°C . was found to be more lethal than crystallization and devitrification even more so. The latter includes both the injury of vitrification upon cooling and the injury of crystallization upon warming. The significance of their quantitative studies, however, awaits further elucidation.

The killing of bacterial cells by cold is clearly not a function of cold *per se*, but actually works in reverse, and is most noticeable at temperatures just below the minimum for growth. We have to turn, then, to the physico-chemical field to seek a possible explanation.

LITERATURE CITED

1. Tressler, D. K., and Evers, C. F., *The Freezing Preservation of Foods* (Avi Publishing Company, Inc., New York, N. Y., 932 pp., 1947)
2. Gortner, W. A., Erdman, F. S., and Masterman, N. K., *Principles of Food Freezing* (John Wiley & Sons, Inc., New York, N. Y., 281 pp., 1948)
3. Brown, H. D., Kunkle, L. E., and Winter, A. R., *Frozen Food Processing and Handling* (S. F. Hinkle & Sons Co., Asheville, Ohio, 300 pp., 1946)
4. Cruess, W. V., *Commercial Fruit and Vegetable Products*, 678-715 (McGraw-Hill Book Company, Inc., New York, N. Y., 906 pp., 1948)
5. Pennington, M. E., and Tressler, D. K., in *The Chemistry and Technology of Food and Food Products*, 3, 1841-1857 (Jacobs, M. B., Ed., Interscience Publishers, Inc., New York, N. Y., pp. 2580, 1951)
6. Prescott, S. C., and Proctor, B. E., *Food Technology*, 443-87 (McGraw-Hill Book Company, Inc., New York, N. Y., 630 pp., 1937)
7. von Loesecke, H. W., *Outlines of Food Technology*, 532-67 (Reinhold Publishing Corp., New York, N. Y., 583 pp., 1949)
8. "Frozen Foods," *The Refrigerating Data Book*, Refrig. Application Vol., Sect. 1, 5-145 (Fitzgerald, G. A., Ed., Am. Soc. Refrig. Engrs., New York, N. Y., 550 pp., 1950)
9. Diehl, H. C., Magness, J. R., Gross, C. R., and Bonney, V. B., *U. S. Dept. Agr. Tech. Bull.*, No. 148, 1-38 (1930)
10. Diehl, H. C., Wiegand, E. H., and Berry, J. A., *U. S. Dept. Agr. Circ.*, No. 53, 1-58 (1939)
11. Joslyn, M. A., and Hohl, L. A., *Agr. Expt. Sta. (Calif.) Bull.*, No. 703, 1-108 (1948)
12. Wiegand, E. H., *Agr. Expt. Sta. (Oregon) Bull.*, No. 278, 1-42 (1931)
13. Woodroof, J. G., *Agr. Expt. Sta. (Ga.) Bull.*, No. 168, 1-23 (1931)
14. Carlton, H., *Agr. Expt. Sta. (Tenn.) Bull.*, No. 173, 1-175 (1941)
15. Tressler, D. J., Evers, C. F., and Long, L., *Into the Freezer and Out* (Avi Publishing Co., Inc., New York, N. Y., 223 pp., 1946)
16. Tressler, D. K., and DuBois, C. W., *Agr. Expt. Sta. (N. Y.) Bull.*, No. 690, 1-60 (1940)
17. Bedford, C. L., Berry, J. A., Boggs, M. M., and Campbell, H., *Agr. Expt. Sta. (Wash.) Bull.*, No. 1-46 (1945)
18. Carlton, H., *Agr. Expt. Sta. (Tenn.) Bull.*, No. 280, 1-16 (1944)

19. Diehl, H. C., and Warner, K. F., *U. S. Dept. Agr. Circ.*, No. 709, 1-62 (1945)
20. Mrak, V. G., *Agr. Expt. Sta. (Calif.) Circ.*, No. 153, 1-21 (1949)
21. Plagge, H. H., and Lowe, B., *Agr. Expt. Sta. (Iowa) Bull.*, No. P46, 486-528 (1942)
22. Winter, J. D., *Agr. Expt. Sta. (Minn.) Bull.*, No. 362, 1-24 (1942)
23. Winter, J. D., and Hustrulid, A., *Agr. Expt. Sta. (Minn.) Bull.*, No. 244, 1-24 (1944)
24. Dawson, E. H., Gilpin, G. L., and Reynolds, H., *U. S. Dept. Agric., Agric. Handbook No. 2*, 1-106 (1950)
25. *U. S. Bur. Agr. Ind. Chem. Circ. Ser.*, A1C-46, 1-16 (1945)
26. Erdman, F. S., *Refrig. Eng.*, **48**, 374-80, 414, 416, 418, 420, 422, 426, 428, 430 (1944)
27. Weil, B. H., and Sterne, F., *Georgia School Technol., State Eng. Expt. Sta., Spec. Rept.*, No. 23, 1-406 (1946)
28. Anderson, B., and Weil, B. H., *Georgia School Technol., State Eng. Expt. Sta., Spec. Rept.*, No. 25, 407-670 (1948)
29. Diehl, H. C., *Ind. Eng. Chem.*, **24**, 661-65 (1932)
30. Joslyn, M. A., *Food Inds.*, **2**, 350-52 (1930)
31. Joslyn, M. A., *Advances in Enzymol.*, **9**, 613-52 (1949)
32. Joslyn, M. A., and Ponting, J. D., *Advances in Food Research*, **3**, 1-44 (1950)
33. Biale, J. B., *Ann. Rev. Plant Physiol.*, **1**, 183-205 (1950)
34. Bedford, R. H., *5th Pacific Sci. Congr. Proc. (Canada)*, **5**, 3715-24 (1933)
35. Luyet, B. J., and Gehenio, P. M., *Life and Death at Low Temperatures* (Biodynamica, Normandy, Mo., 341 pp., 1940)
36. Joslyn, M. A., and Cruess, W. V., *Fruit Products J.*, **8**(7), 9-12; **8**(8), 9-12 (1929)
37. Joslyn, M. A., and Marsh, G. L., *Agr. Expt. Sta. (Calif.) Bull.*, No. 551, 1-40 (1933)
38. Sachs, J., *Landw. Vers.-Sta.* No. 5, 167-201 (1860)
39. Müller-Thurgau, R., *Landw. Jahrb.*, **9**, 133-89 (1880); **15**, 453-610 (1886)
40. Molisch, H., *Untersuchungen über das Erfrieren der Pflanzen*, 1-73 (G. Fischer, Jena, Germany, 73 pp., 1897)
41. Matruchot, L., and Molliard, M., *Rev. gén. botan.*, **14**, 401-19, 463-82, 522-28 (1902)
42. Chandler, W. H., *Agric. Expt. Sta. (Missouri) Research Bull.*, No. 8, 143-309 (1908)
43. Chandler, W. H., *Fruit Products J.*, **12**(2), 50-51, 57 (1932)
- 43a. Chandler, W. H., *Am. J. Bot.*, **18**, 892-94 (1931)
44. Chandler, W. H., and Hildreth, A. C., *Proc. Am. Soc. Hort. Sci.*, **33**, 27-35 (1936)
45. Maximow, N. A., *Ber. Deut. Botan. Ges.*, **30**, 52-65, 243-305, 504-16 (1912); *Jahrb. wiss. Botan.*, **53**, 327-40 (1914)
46. Finnegan, W. J., *Food Packer*, **30**(8), 29-32; **30**(9) 27-29; (No. 10), 32-33 (1949); *Refrig. J.*, **2**, 1107-8, 1120-21, 1128-30 (1949)
47. Dorsey, Noah Ernest, *Properties of Ordinary Water-Substance in All Its Phases: Water Vapor, Water and All the Ices* (Reinhold Publishing Corp., New York, N. Y., 673 pp., 1940)
48. Luyet, B. J., *Biodynamica*, **1**, No. 29, 1-14 (1937)
49. Luyet, B. J., and Gehenio, P. M., *Biodynamica*, **1**, No. 30, 1-23 (1937)
50. Luyet, B. J., and Condon, H. M., *Biodynamica*, **2**, No. 37, 1-8 (1938)
51. Luyet, B. J., and Thoenes, G., *Science*, **88**, 284-85 (1938)
52. Gehenio, P. M., and Luyet, B. J., *Biodynamica*, **2**, No. 55, 1-22 (1939)

53. Luyet, B. J., and Galos, G., *Biodynamica*, **3**, No. 65, 157-69 (1940)
54. Gehenio, P. M., *Biodynamica*, **3**, 347-52 (1941)
55. Woodroof, J. G., *Agr. Expt. Sta. (Georgia) Bull.*, No. 168, 1-23 (1931)
56. Kaloyereas, S. A., *Food Research*, **12**, 419-27 (1947)
57. Kethley, T. W., Cown, W. B., and Bellinger, F., *Food Inds.*, **21**, 179-82, 286-87 (1949)
58. Woodroof, J. G., *Agr. Expt. Sta. (Georgia) Bull.*, No. 201, 3-46 (1938)
59. Woodroof, J. G., *Science*, **89**, 87-88 (1939)
60. MacArthur, M., *Fruit Products J.*, **24**, 238-40 (1945)
61. MacArthur, M., *Sci. Agr.*, **28**, 166-74 (1948)
62. Lee, F. A., Gortner, W. A., and Whitcombe, J., *Ind. Eng. Chem.*, **38**, 341-46 (1946)
63. Lee, F. A., and Gortner, W. A., *Refrig. Eng.*, **57**, 148-51, 184-87 (1949)
64. Lee, F. A., Gortner, W. A., and Whitcombe, J., *Food Technol.*, **3**, 164-69 (1949)
65. Lebeaux, J. M., *Refrig. Eng.*, **54**, 531-33 (1947)
66. Weier, T. E., and Stocking, R., *Advances in Food Research*, **2**, 297-342 (1949)
67. Hohl, L. A., *Food Technol.*, **2**, 158-62 (1948)
68. Joslyn, M. A., and Marsh, G. L., *Fruit Products J.*, **12**, 203-8 (1933)
69. Lee, F. A., and Johannesen, G. A., *Food Technol.*, **5**, 263-65 (1951)
70. Cook, W. H., and Lusena, C. V., *Proc. 8th Intern. Congr. Refrig.* (In press)
71. Nord, F. F., *Ergeb. Enzymforsch.*, **2**, 23-49 (1933)
72. Nord, F. F., *Trabajos 9th Cong. Intern. de Químico Pina y Aplicada*, **5**, 1-17 (1933)
73. Nord, F. F., *Protoplasma*, **21**, 116-28 (1934)
74. Nord, F. F., Leichter, H., and Umbach, G., *Z. Elektrochem.*, **43**, 682 (1937)
75. Leichter, H., and Nord, F. F., *Biochem. Z.*, **295**, 226-36 (1937)
76. Holzapfel, L., and Nord, F. F., *Naturwissenschaften*, **26**, 283-84 (1938)
77. Holzapfel, L., *Kolloid.-Z.*, **85**, 272-78 (1938)
78. Leichter, H., *Proc., 8th Intern. Congr. Refrig.* (In press)
79. Gorke, H., *Landw. Vers. Sta.*, **65**, 149-60 (1907)
80. Harvey, R. B., *J. Agr. Research*, **15**, 83-111 (1918)
81. Pickett, T. A., *Ind. Eng. Chem.*, **24**, 353-54 (1932)
82. Holzapfel, L., and Nord, F. F., *Biodynamica*, **3**, 1-9 (1940)
83. Daughters, M. R., and Glenn, D. S., *Refrig. Eng.*, **52**, 137-40 (1946)
84. Howard, L. B., and Campbell, H., *Food Inds.*, **18**, 674-77 (1946)
85. Talburt, W. F., and Legault, R. R., *Food Technol.*, **4**, 286-91 (1950)
86. Friess, H., *Food Technol.*, **2**, 191-200 (1948)
87. Joslyn, M. A., *Food Technol.*, **3**, 8-14 (1949)
88. Caul, J. F., Sjöström, L. B., and Turner, W. P., *Quick Frozen Foods*, **12**, 54-58 (1950)
89. Caul, J. F., and Sjöström, L. B., *Quick Frozen Foods*, **13**(10), 59-61, 111 (1951)
90. Rabak, W., and Diehl, H. C., *Western Canner and Packer*, **36**(4), 45 (1944)
91. Brekke, J. F., and Talburt, W. F., *Food Technol.*, **4**, 383-86 (1950)
92. Buck, R. E., Baker, G. L., and Mottern, H. H., *Food Inds.*, **16**, 100-2, 134 (1944)
93. Wegener, J. B., Baer, B. H., and Rodgers, P. D., *Quick Frozen Foods*, **14**(1), 50-51 (1951)
94. Lee, F. A., Fenton, F., and Stevens, H. B., *Food Technol.*, **5**, 114-16 (1951)
95. Joslyn, M. A., *J. Sci. Food Agr.*, **2**, 289-94 (1951)
96. Tressler, D. K., *Ind. Eng. Chem.*, **24**, 682-85 (1932)
97. Mergentime, M., Wiegand, E. H., *Fruit Products J.*, **26**, 72-80, 89, 91-92 (1946)

98. Lee, F. A., and Wagenknecht, A. C., *Food Research*, **16**, 239-44 (1951)
99. Gutterman, B. M., Lovejoy, R. D., and Beacham, L. M., *J. Assoc. Offic. Agr. Chemists*, **34**, 231-32 (1951)
100. Barker, J., and Morris, T. N., *Dept. Sci. Ind. Research (Brit.) Comm. Repts.* 67 (1930); 78 (1931); 79 (1932)
101. Diehl, H. C., Dingle, J. H., and Berry, J. A., *Food Inds.*, **5**, 300-1 (1933)
102. Diehl, H. C., and Berry, J. A., *Proc. Am. Soc. Hort. Sci.*, **30**, 496-500 (1933)
103. Joslyn, M. A., *Agr. Expt. Sta. (Calif.) Circ.*, No. 320, 1-35 (1930)
104. Joslyn, M. A., and Marsh, G. L., *Science*, **72**, 174 (1933)
105. Kohman, E. F., *Canner*, **68**, 147-49 (1929)
106. Lindquist, F. E., Dietrich, W. C., Masure, M. P., and Boggs, M. M., *Food Technol.*, **5**, 198-99 (1951)
107. Campbell, H., *Quick Frozen Foods*, **12**(7), 129-32 (1950)
108. Kiermeier, F., *Proc. 8th Intern. Congr. Refrig.* (In press)
109. Lerner, A. B., and Fitzpatrick, T. B., *Physiol. Revs.*, **30**, 91-126 (1950)
110. Guadagni, D. G., Sorber, D. G., and Wilbur, J. S., *Food Technol.*, **3**, 359-64 (1949)
111. Kertesz, Z. I., *State Agr. Sta. (N. Y.) Bull.*, No. 219, 1-14 (1933)
112. Johnson, G., Foreman, E. M., and Mayer, M. M., *Food Technol.*, **4**, 237-41 (1950)
113. Johnson, G., Mayer, M. M., and Johnson, I. K., *Food Research*, **16**, 169-80 (1951)
114. Clark, L. M., and Levy, W. J., *J. Sci. Food Agr.*, **1**, 213-14 (1950)
115. Bate-Smith, E. C., and Westall, R. G., *Biochim. et Biophys. Acta*, **4**, 427-40 (1950)
116. Dimick, K. P., Ponting, J. D., and Makower, B., *Food Technol.*, **5**, 237-41 (1951)
117. Caldwell, J. S., Lutz, J. M., and Moon, H. H., *Food Inds.*, **4**, 402-5 (1932)
118. Caldwell, J. S., Lutz, J. M., and Moon, H. H., *Proc. Am. Soc. Hort. Sci.*, **29**, 282-86 (1932)
119. Caldwell, J. S., Lutz, J. M., Moon, H. H., and Myers, A. T., *Fruit Products J.*, **12**, 366-71 (1933)
120. Lutz, J. M., Caldwell, J. S., Moon, H. H., and Myers, A. T., *Fruit Products J.*, **13**, 7-10 (1933)
121. Diehl, H. C., Pentzer, W. T., Berry, J. A., and Asbury, C. E., *Western Canner and Packer*, **26**(5), 31-33; **26**(6), 39-41; **26**(7), 33-35; **26**(8), 43-44 (1934)
122. Caldwell, J. S., Lutz, J. M., Moon, H. H., and Culpepper, C. W., *U. S. Dept. Agr. Tech. Bull.*, No. 731, 1-72 (1940)
- 123a. Schwartz, C. D., and Diehl, H. C., *Agr. Expt. Sta. (Washington)*, U. S. Bur. Chem. and Soils, Prelim. Rept., Mimeo. (1936)
- 123b. Schwartz, C. D., Boggs, M. M., Campbell, H. T., and Diehl, H. C., *Agr. Expt. Sta. (Washington)*, U. S. Bur. Chem. and Soils, Second Rept., Mimeo. (1937)
124. *Agr. Expt. Sta. (Oregon) Progress Rept.*, U. S. Bur. Chem. and Soils and U. S. Plant Ind., Mimeo. (1937)
125. Kramer, A., and Smith, H. R., *Food Packer*, **27**(8), 56-9 (1946)
126. Kramer, A., and Smith, H. R., *Food Technol.*, **1**, 527-39 (1947)
127. Kramer, A., Guyer, R. B., and Ide, L. E., *Food Packer*, **30**(8), 41-44 (1949)
128. Kramer, A., Haut, I. C., Scott, L. E., and Ide, L. E., *Proc. Am. Soc. Hort. Sci.*, **53**, 411-25 (1949)
129. Kramer, A., *Food Technol.*, **5**, 265-69 (1951)
130. Lee, F. A., DeFelice, D., and Jenkins, R. R., *Ind. Eng. Chem., Anal. Ed.*, **14**, 240-41 (1942)

131. Lee, F. A., and DeFelice, D., *Canner*, **94**(26), 11-13, 24 (1942)
132. Lee, F. A., *Food Research*, **5**, 161-66 (1940); **8**, 249-53 (1943)
133. Lee, F. A., *Agr. Expt. Sta. (N. Y.) Bull.*, No. 729, 1-12 (1948)
134. Makower, R. U., *Food Technol.*, **4**, 403-8 (1950)
135. Heid, J. L., and Makower, B. W., *Food Technol.*, **5**, 347-49 (1951)
136. Gould, W. A., Krantz, Jr., F. A., and Mavis, J., *Food Technol.*, **5**, 175-79 (1951)
137. Gould, W. A., *Agr. Expt. Sta. (Ohio) Bull.*, No. 701, 1-34 (1951)
138. Robinson, W. B., Lee, F. A., Slate, G. L., and Pederson, C. S., *Agr. Expt. Sta. (N. Y.) Bull.*, No. 726, 1-14 (1947)
139. Campbell, H., and Diehl, H. C., *Western Canner and Packer*, **32**(10), 48-50; **32**(11), 41-53 (1940)
140. Makower, R. U., and Ward, A. C., *Food Technol.*, **4**, 46-49 (1950)
141. Boggs, M. M., Campbell, H., and Schwartze, C. D., *Food Research*, **7**, 272-87 (1942); **8**, 505-15 (1943)
142. Nielsen, J. P., Campbell, H., Bohart, C. S., and Masure, M. P., *Food Inds.*, **19**, 305-8, 432, 434, 479-82, 580 (1947)
143. Heiss, R., *Proc. 8th Intern. Congr. Refrig.* (In press)
144. Finnegan, W. J., *Ice and Refrig.*, **94**(1), 45-48 (1938)
145. Finnegan, W. J., and Carter, T. F., *Refrig. Eng.*, **54**, 132-34, 174, 176 (1947)
146. Joslyn, M. A., and Marsh, G. L., *Ind. Eng. Chem.*, **22**, 1192-97 (1930)
147. Joslyn, M. A., and Marsh, G. L., *Refrig. Eng.*, **24**, 81-8 (1932)
148. Joslyn, M. A., and Marsh, G. L., *Refrig. Eng.*, **24**, 214-24, 234, 236, 239 (1932)
149. Finnegan, W. J., *Refrig. Eng.*, **37**, 381-383 (1939)
150. Nicholas, J. E., *Agr. Expt. Sta. (Penn.) Bull.*, No. 471, 1-20 (1945)
151. Phillips, W. R., *Refrig. Eng.*, **52**, 401-3 (1947)
152. Short, B. E., *Univ. Texas Pub.*, No. 4432, 1-26 (1944)
153. Kethley, T. W., Cown, W. B., and Bellinger, F., *Refrig. Eng.*, **58**, 49-50 (1950)
154. Woolrich, W. R., *Univ. Tenn. Eng. Expt. Sta. Bull.*, No. 11, 1-18 (1933)
- 154a. Riedel, L., *Mitt. Kältetechn. Inst. Reichforschungsanst. Lebensmittel frischhalt. Tech. Hochschule Karlsruhe*, 3-47 (1948); *Chem. Zentr.*, **II**, 1266-67 (1948)
- 154b. Riedel, L., *Chem.-Ing.-Tech.*, **23**, 321-24 (1951)
- 154c. Riedel, L., *Chem.-Ing.-Tech.*, **21**, 340-41 (1949)
- 154d. Riedel, L., *Z. Lebensm. Untersuch. u. Forsch.*, **89**, 289-99 (1949)
- 154e. Riedel, L., *Refrig. Eng.*, **59**, 670-73 (1951)
155. Haines, R. B., *J. Soc. Chem. Ind.*, **50**, 223-7 T (1931)
156. James, H. L., *Fruit Products J.*, **12**, 110-13 (1932)
157. Wallace, G. I., and Tanner, F. W., *Fruit Products J.*, **13**, 52-56, 109-13 (1933)
158. Berry, J. A., and Magoon, C. A., *Phytopathology*, **24**, 780-96 (1934)
159. Haines, R. B., *Proc. Brit. Assoc. Refrig.*, **33**(2), 62-9 (1936-37)
160. Haines, R. B., *Ice and Refrig.*, **93**, 199-200 (1937)
161. Haines, R. B., *Proc. Roy Soc. (London)*, [B]**124**, 451-63 (1938)
162. Prescott, S. C., and Tanner, F. W., *Food Research*, **3**, 189-97 (1938)
163. Pederson, C. S., *Food Research*, **12**, 429-38 (1947)
164. Haines, R. B., *J. Hyg.*, **34**, 277-82 (1934)
165. Berry, J. A., *J. Bact.*, **26**, 459-70 (1933)
166. Van Eseltine, W. P., Nellis, L. F., Lee, F. A., and Hacker, G. J., *Food Research*, **13**, 271-80 (1948)
167. Weiser, R. S., and Osterud, C. M., *J. Bact.*, **50**, 413-39 (1945)
168. Weiser, R. S., and Hargiss, C. O., *J. Bact.*, **52**, 71-79 (1946)
169. Prescott, S. C., Bates, P. K., and Highlands, M. E., *Am. J. Pub. Health*, **22**, 257-62 (1932)
170. Bedford, R. H., *Biol. Board Can. Progress Repts.*, No. 22, 14-15 (1934)
171. Bigelow, S. L., and Rykenboer, E. A., *J. Phys. Chem.*, **21**, 474-521 (1921)

PHYSIOLOGY OF VIRUS DISEASES¹

BY F. C. BAWDEN AND N. W. PIRIE

Rothamsted Experimental Station, Harpenden, Hertfordshire, England

Review articles on viruses and virus diseases are already included in a wide variety of periodicals. There is no need, because of this, to apologise for their inclusion in the *Annual Review of Plant Physiology*, but the appearance of the first article on the subject in any new surroundings is an occasion to consider the sort of material most appropriate to the surroundings.

There were never valid reasons for distinguishing sharply between viruses that attack plants and those that attack animals. The distinction has become even more diffuse with the demonstration that clover club leaf (1) and aster yellows (2) viruses multiply in their insect vectors. Nevertheless, work on animal and bacterial viruses will be excluded unless the results can be applied to virus diseases of higher plants. These diseases are most plausibly regarded as metabolic disturbances of the host, which produce, among other things, more material that can induce similar metabolic disturbances in other hosts. Few of the known virus diseases have been studied chemically in any detail, but each has involved an aberration of nucleoprotein metabolism. It is reasonable, therefore, always to seek anomalies in the nucleoproteins, but the search should not stop there, for it would be premature to make the restrictive generalisation that all virus diseases are transmissible aberrations of nucleoprotein metabolism. Any biochemical or physiological studies on infected plants may contribute to an understanding of virus diseases; conversely, knowledge of the plant's reaction to infection may contribute to plant physiology. However, limits must be drawn somewhere, and many subjects usually considered in reviews on viruses will be excluded. For example, the physicochemical properties of virus preparations, although their study bulks large in recent work, and studies on transmission by insect and other vectors, are more suitable for other journals. The movement of viruses in the plant is germane, but is not included because it has recently been reviewed (3).

The metabolic processes and changes involved in virus infections are highly appropriate for treatment in this review, but their study has, unfortunately, been neglected. It is difficult to understand why. The problems are admittedly complex, but the infectivity of virus particles effectively "labels" them, so that they can be specifically assayed at low concentrations. This makes them convenient subjects for work on such problems as translocation and the factors that affect at least one kind of protein synthesis. There is, too, much evidence that the ability of viruses to enter, multiply in, and affect the normal working of susceptible plants, depends greatly on the physiologi-

¹ The survey of the literature pertaining to this review was completed in September, 1951.

cal condition of the plants. Indeed, many of the apparent contradictions in the literature on virus diseases originate in this fact. Viruses have been most extensively studied in leaves. The responsiveness of the leaf to changes in environmental and cultural conditions is a troublesome complication when reproducible results are sought and a uniform starting material is needed. This very responsiveness, however, makes the higher plants particularly suitable systems in which to study the factors that influence the reactions between viruses and cells, because it permits their study under a much wider range of conditions than is possible with most other kinds of organisms.

Susceptibility is a term that is used loosely to cover a number of different concepts. First, the ease with which a plant becomes infected when a given inoculum is applied in a given manner; it can be shown either by the proportion of a group of plants that becomes infected or by the number of lesions that appears on a leaf. This is relatively unambiguous and can be measured with some accuracy; we shall refer to it as susceptibility to infection. Secondly, there is severity of reaction, which is a more aesthetic concept and depends on a judgement of the extent to which a plant has been crippled. Its use also involves the difficulty that an effect that is locally severe may prevent further spread. A virus causing mosaic symptoms may produce more generalised damage than one that kills cells. In the limit, there is the possibility that a virus could so abruptly stop the activities of a single cell that there would be little virus multiplication. This would be the maximum of local severity, but would not affect the plant as a whole and would be impossible, practically, to observe. Thirdly, there is the quantity of virus produced, which, at first sight, is easily assessed, for infective extracts can be made from plants infected with many of the viruses with which we are concerned. However, although an essential property of a virus, infectivity is capricious. Fortunately, most normal plant proteins seem to be relatively poor antigens, whereas the plant viruses that have been studied are very active in producing antibodies when injected into animals. Antisera provide a rapid, and probably the most trustworthy, method for measuring the amount of anomalous material present in both the fluid and solid parts of infected leaves.

The establishment of infection may depend as much on the physiological condition of the host cell as on the state of the virus particles. Infectivity is often regarded as a property intrinsic to the particles and sharp distinctions are drawn between infective and noninfective particles. This is unjustified, because particles that fail to infect a potentially susceptible host under one set of conditions may do so under another. The local-lesion method is widely used to assay plant viruses and it can give reliable results provided that the inocula are identical in all respects except virus content and inoculations are made to uniform test plants. When these provisos do not apply, lesion counts may give wholly misleading comparisons of the numbers of potentially infective particles in different inocula. Many attempts have been made to interpret the relationships between virus concentration and lesion counts by analogies with plating bacteria. It has been concluded that infection occurs

because of chance encounters between single virus particles and infection sites of a uniform type (4 to 8), although not all the evidence has favoured this conclusion. On this view, variations in susceptibility to infection simply reflect differences in the numbers of sites at which infection can occur. The only statistical examination (9) of dilution curves has shown that this conclusion is invalid and that dilution curves are compatible with the hypothesis that individual infection sites vary in their susceptibility so that different quantities of virus are needed to initiate infection at different sites. This does not mean that single particles do not cause infections, but more likely that the probability of a particle doing so varies from site to site (10). The reason for this variability is unknown, but different cells may contain varying amounts of materials that combine with virus particles and interfere with their establishment on essential receptors.

CHANGES IN HOST METABOLISM THAT INFLUENCE VIRUS INFECTIONS

A virus infection can be studied and assessed in two ways. First, we can record changes in the appearance or behaviour of the host, such as the development of macroscopic or microscopic symptoms or resistance to a second infection. Secondly, we can demonstrate that extracts of plants contain material with the infective, serological, or physicochemical properties that have come to be associated with the viruses. It is important to realise that the two methods give essentially different types of information. The first tells how many plants, and approximately what proportion of each plant, have been affected; the second tells how much anomalous material can be extracted by the methods used. There is no general relationship between the two. Thus, the most severe symptoms are not necessarily shown by plants which provide extracts containing the largest amount of anomalous material. There are several reasons for this. A necrotic lesion is severe locally, but much of the leaf may be unaffected; most of the anomalous material is often not readily extractable from the insoluble, structural parts of leaves (11). With either condition, a simple measurement of the amount of anomalous material in an extract, made in some arbitrary way, tells us very little; even a thorough assay of all that can be released by enzymes does not give information about local intensity of virus multiplication, and this is the fundamental quantity. The later manifestations of infection depend on the movement of the virus through the host, the manner in which it affects host metabolism, whether it be by blocking enzymes or sequestering metabolites, and, perhaps, on the rate at which it is destroyed or inactivated by the host. None of these features is obviously correlated with another, nor with the rate of virus multiplication. With these reservations in mind, we shall survey some of the observations that have been made on the effects of changes in the physiological state of the host on the manifestations of virus infection.

Seasonal changes.—Everyone who maintains plants throughout the year in latitudes with large seasonal changes will be familiar with effects of environment on symptomatology. It is obvious, too, that seasonal changes af-

fect different virus diseases differently; some, particularly the yellows type (for example, potato leaf roll and sugar beet yellows) are severe during summer but slight during winter, whereas many of the mosaic type viruses (for example, tobacco etch, potato X and Y, and tomato bushy stunt) cause much more severe symptoms in winter than in summer. An extreme example of seasonal behaviour is given by *Phaseolus vulgaris*, vars. Prince and Bountiful, when inoculated with cucumber mosaic virus; during summer this host is immune, whereas during winter it produces countable necrotic local lesions suitable for quantitative assays (12). Despite the many and varied effects that have been described, the subject has received little critical attention. Most references are to the appearance of infected plants; and susceptibility has generally been used as though it were synonymous with sensitivity. Severity of reaction, however, is only one of the qualities included in the general term susceptibility. There is no *a priori* reason why conditions that make a plant less sensitive to the presence of a virus should also render it less liable to contract infection or less suitable to maintain a high virus content. Seasonal differences may also affect the extractibility of viruses and their behaviour after extraction; for example, extracts produced by finely grinding fibre of plants infected with tomato bushy stunt virus in the summer contain more material that interferes with the precipitation of the virus by its antiserum than do extracts produced during winter (13).

Illumination.—Many of the seasonal effects noted under glass result from changes caused by growing the plants under different light intensities; thus, winter reactions can be obtained in summer by shading. It is characteristic of the leaves of plants affected with potato leaf roll or sugar beet yellows to contain more carbohydrate than normal leaves, and the reduction in symptoms produced by reducing light intensity is conveniently explained by simple reduction of photosynthesis. There is, however, no such easy explanation for the disappearance of the bright yellow mottling when plants infected with *Abutilon* mosaic (14), tomato aucuba mosaic (15), and cucumber 4 (16) viruses are shaded; nor for the fact that shading branches of cherry trees suffering from little cherry permits them to produce a crop of normal fruit (17).

Variations in light intensity affect not only the symptoms shown by systemically infected plants, but also the readiness with which leaves become infected. If, during summer, *Nicotiana glutinosa*, tobacco, or French bean plants are raised under one-third the normal light intensity, by shading with muslin, their susceptibility to infection, as measured by counts of local lesions, by tomato bushy stunt, tobacco mosaic, and tobacco necrosis viruses, is increased by factors of from 5 to 10 (18). The interpretation of such effects in terms of altered plant physiology is complicated by the fact that leaves have to be injured before they can become infected. Shading leaves increases their fragility. The increased susceptibility may largely reflect the fact that shaded leaves are more readily injured when rubbed with inocula. There is evidence, however, that changes other than those altering the toughness of the cuticle are involved. Susceptibility to infection can be increased by keep-

ing plants in darkness for only about 24 hr. before inoculation; this treatment does not obviously increase the fragility of the leaves (19). A preconditioning of this type may so increase susceptibility that plants otherwise immune from infection by the mechanical inoculation of sap become susceptible, for example, ground-nut plants with rosette virus (20) and potato plants with potato yellow dwarf virus (21).

Once the leaves have been inoculated, shading or placing in darkness does not increase the number of lesions. On the contrary, if plants inoculated with tobacco necrosis or tomato bushy stunt viruses are immediately put into the dark, they usually produce fewer lesions than if they are kept in the light (19). A more dramatic effect, which may have a similar basis, is obtained if the virus preparation is first partially inactivated by irradiation with ultraviolet light, for then many more lesions are produced on illuminated leaves (22). Clearly, changes in the resistance of the epidermis to injury do not cause this effect, for it is obtained by treating leaves after they have been inoculated. Nor is it a direct effect of light on the virus, for exposing the ultraviolet-treated virus to visible light *in vitro* does not increase infectivity. Although the short-lived intermediates in photosynthesis may influence infection and virus multiplication, this effect of light need not be connected with photosynthesis; a similar phenomenon, which has been called "photoreactivation," occurs with some fungus spores, bacteria, and bacterial viruses, systems neither normally nor necessarily exposed to light.

No satisfactory explanation of photoreactivation has been given, but many enzymes and coenzymes are coloured and so can be affected by visible light. Exposure of leaves to light does more than cause the photosynthesis of carbohydrates; for example, it destroys growth substances (23) and activates glycolic acid oxidase (24). Presumably some such mechanism in the host either restores to its previous state some virus changed by ultraviolet or creates an environment in which the changed virus can multiply. Local-lesion counts measure the number of points at which multiplication proceeds extensively enough to cause a visible symptom. There may be types of inactivation that allow virus particles to start the process while being able to complete it only in an especially favourable environment.

Although photoreactivation occurs in many different biological systems, not all plant viruses show it. Leaves inoculated with ultraviolet-treated tobacco mosaic virus produce proportionally no more lesions when illuminated than do leaves inoculated with unirradiated virus. Nor does illumination after inoculation increase the numbers of lesions produced by inocula of all unirradiated viruses; exposing leaves inoculated with tomato spotted wilt virus to sunlight reduces the number of lesions (25) and cucumber mosaic virus produces more lesions in French bean plants if these are kept in darkness both before and after inoculation than if they are illuminated (12).

Temperature.—Differences in the temperature at which plants are kept have effects even more striking than those caused by differences in light intensity. The effect most often noted is a reduction in severity, sometimes

complete "masking," of symptoms with increasing temperature. This has been described for many mosaic diseases (26, 27), but the temperature at which masking occurs differs with different diseases. With tobacco mosaic virus, masking occurs mainly, if not exclusively, because high temperatures preferentially select avirulent strains (28). There is no evidence of this with other viruses and it seems that the effect must reflect some change in the physiology of the host that interferes with virus multiplication or the formation of products with which the viruses usually interact to cause symptoms. With most diseases, no attempt has been made to correlate the severity of symptoms with the quantity of virus, but with cabbage black ringspot and cauliflower mosaic viruses the symptoms are most pronounced at temperatures at which expressed sap also has the greatest infectivity (29, 30). These optimal temperatures are not determined solely by the virus, but depend also on the species of the host; black ringspot virus, for example, produces the most severe symptoms in cabbage when the air temperature is around 28°C. and in *Nicotiana glutinosa* around 18°C.

The symptoms caused by tobacco mosaic virus in *N. glutinosa* also depend on the air temperature: at 25°C. or lower, infection produces discrete necrotic local lesions; above 28°C., the lesions, although still necrotic, are larger and tend to coalesce; and above 35°C., there is no necrosis but chlorosis and the virus invades the plant systemically (31).

The effects of heat show most dramatically in the successes that have been achieved by heat therapy. Peach trees have been freed from various viruses by keeping at temperatures of 35°C. for many days or by treating dormant wood for shorter times at higher temperatures (32 to 35); *Vinca rosea* has been freed from aster yellows, cranberry false-blossom, and potato witches' broom viruses (36, 37, 38), and potato tubers from witches' broom (37) and leaf roll viruses (39). The effect of heat in freeing tissues from infecting viruses is usually attributed to the direct inactivation of the virus. This may be so, and when the effect is obtained by short exposures to high temperatures it probably is, but another explanation is equally plausible for the cures obtained by prolonged exposures around 35°C. Although some viruses are exceptionally resistant to heat *in vitro*, it is reasonable to expect that most, if not all, undergo a normal inactivation *in vivo*. If this is so, the virus content of a tissue depends on the equilibrium established between multiplication and this normal inactivation. It has been suggested (40) that reducing the rate of multiplication of potato virus Y below that necessary to counter normal inactivation is responsible for the disappearance of this virus in plants that are infected with severe etch virus. It may be that a rise in temperature acts similarly. The apparent disappearance of a virus at 35°C. *in vivo* may be failure of synthesis rather than heat inactivation.

Nothing has been published on the influence of heat on the susceptibility of plants to infection, but recent work by Kassanis (41) suggests that it is considerable. *N. glutinosa* and French bean plants kept at 35°C. for a day or so before inoculation with tobacco mosaic, tomato bushy stunt, and tobac-

co necrosis viruses, behave similarly to plants kept in the dark and produce more lesions than control plants. Placing the plants at 35°C. after inoculation considerably reduces the numbers of lesions.

Summarising the effects of changes in light and temperature, it can be said that low light intensities and high temperatures produce conditions in leaves that favour the initiation of infection, but that its maintenance is favoured by stronger light and lower temperature. The biochemical mechanism of these effects is obscure, but they illustrate the intricacy of the relationship between virus and host and the inadequacy of the idea that this relationship resembles that of a bacterium and an agar plate.

Nutrition.—Light and temperature affect the composition of the leaf but in, at present, incompletely known and unpredictable ways; some changes in the environment to which the roots are exposed affect the composition of the leaves directly, but the effects on susceptibility to infection are smaller. Only tobacco mosaic virus has been studied and the results with it are conflicting. Spencer (42, 43) concluded that growth rate and susceptibility to infection were not correlated; whereas nitrogen specifically increased susceptibility, even when given in such large amounts that it restricted growth, phosphorus increased susceptibility only while also increasing growth, and potassium decreased susceptibility at levels that increased growth. Bawden & Kassanis (44), by contrast, found that both phosphorus and nitrogen increased susceptibility to infection only when applied in amounts that also increased growth and that conditions optimal for growth approximated closely to those at which most local lesions were produced. The main effect was due simply to the increased leaf area on which infection could occur, but within certain ranges increases of both phosphorus and nitrogen also increased the numbers of lesions produced per unit area of leaf.

Spencer (45 to 49) also concluded that nitrogen specifically increased the multiplication of tobacco mosaic virus and increased its concentration in sap from systemically infected plants, even when added in amounts that inhibited growth. The magnitude of the increases recorded differ widely in different papers. Spencer (46, 48, 49) further concluded that the virus from nitrogen-deficient plants was, weight for weight, less infective than virus from plants supplied with abundant nitrogen. Bawden & Kassanis (50) found that the effects of both nitrogen and phosphorus on virus production were correlated with effects on plant growth. Combined supplements of both nutrients to tobacco plants growing in soil doubled the virus concentration in sap and increased the total virus per plant by factors up to 40; nitrogen alone increased neither growth nor virus concentration. Potassium slightly reduced the virus content of sap, although it usually increased plant size and the total amount of virus per plant. Despite the differences in virus content produced by these different fertilizer treatments, the ratio between the amount of virus liberated in the expressed sap and that remaining in the fibre was relatively constant at 1:2.

Bawden & Kassanis found no consistent differences between the infectivity, per unit weight, of virus produced in plants when different combinations of fertilizers were supplied. As an explanation of this discrepancy from Spencer's results, they suggested that virus synthesised under other conditions might have different infectivities towards different host species, for their comparisons were made on *N. glutinosa* whereas Spencer's were on Golden Cluster French bean. Chessin (51) states that this is so and that tobacco mosaic virus from nitrogen-deficient plants was as infective as virus from plants adequately supplied when assayed on *N. glutinosa*, but produced only one-third as many lesions on beans. That changes in tobacco mosaic virus may differentially affect its ability to infect these two hosts is also suggested by the behaviour of carbobenzyloxy, chlorobenzoyl, and benzenesulfonyl derivatives, which were found to infect *N. glutinosa* as readily as untreated virus but to be less infective for beans (52).

The amount of nitrogen supplied to systemically infected plants also affects the severity of symptoms. With diseases as different in type as tobacco mosaic (50) and potato leaf roll (53), increasing the nitrogen supply reduces the severity of symptoms, whereas with onion yellow dwarf (54) increasing nitrogen increases the severity.

The differences in susceptibility to infection shown by whole plants or individual leaves of different ages may also in part reflect changes in nutritional state. In general, young vigorously growing plants are more easily infected than older ones and often become systemically invaded by viruses that are localised in older plants. The effect of increasing age in conferring resistance is shown strikingly by French bean and tobacco necrosis viruses (19) and by *N. glutinosa* and tomato bushy stunt virus (55). At the time when the first-formed leaves of French bean are suitable for local-lesion assays during summer, an increase in age of three days may reduce the numbers of lesions produced by a given inoculum to one-fifth. An inoculum of tomato bushy stunt virus that will produce many lesions on the upper leaves of *N. glutinosa* in the 10 to 12 leaf stage will produce only few on the middle leaves and none on the lowest; this cannot be attributed to a hardening of the epidermis with increasing age for tobacco mosaic virus produces more lesions on the middle and lower leaves than on the upper. An effect that may be partly explicable by changes in leaf anatomy is the difference in the numbers of local lesions obtained on leaves from plants supplied with different amounts of water. Tobacco plants kept dry are less susceptible to infection than plants kept wet, but the sap from leaves with local lesions has a greater virus content when the leaves are from plants that have been kept dry. These leaves are somewhat smaller than the wet leaves but not by a factor large enough to account for the difference in virus content. This again suggests that conditions that favour the initiation of infection do not necessarily favour virus multiplication (56).

Miscellaneous agents.—Substances that combine with viruses *in vitro* and inhibit their infectivity are common (57) and many such occur in leaf

extracts. Whether these play any part *in vivo* is uncertain, for plants that contain them are susceptible to viruses. It is of interest that, when mixed with viruses *in vitro*, they cause less inhibition when inoculations are made to plants which contain them than to those that do not (12, 58, 59). The quantitative effect of such powerful inhibitors as ribonuclease (60) also seems to be determined less by the specific virus than by the host plant to which inoculations are made, which may mean that different plants vary in their ability to split the combination between viruses and different inhibiting substances. There is no evidence whether the differences in susceptibility to infection caused by varying light, temperature, or nutrition are occasioned by changes in the amount of inhibitors produced in leaves.

Many attempts have been made to influence virus multiplication by antibiotics and other agents. With some animal and bacterial viruses, virus multiplication has been impeded without preventing the multiplication of the host cells. In higher plants the problem is at first sight simpler, because virus multiplication occurs mainly in cells that, although they may be increasing in size, have ceased to divide. It is, however, more difficult to get materials into or in close proximity with infected cells. Only with the X disease of peach and mosaic of carnation have successful chemotherapeutic treatments been claimed. Stoddard (61, 62) states that infected peach buds can be freed from the virus by soaking in aqueous solutions of quinyhydrone, urea, or sodium thiosulphate, and that infected buds grow into healthy shoots if grafted on to healthy plants that have been injected or watered with solutions of calcium chloride, sulphanilamide, or hydroquinone. Soaking carnation cuttings in solutions of calcium chloride or zinc sulphate is also stated to free them from mosaic virus, but the tests for the presence of virus seem to have been made solely by fluorescence in ultraviolet (63).

Various other treatments have been reported to interfere with the normal course of infection and virus multiplication. Immersing leaves in or spraying them with dilute solutions of sodium cyanide (64), thiamine or aniline (65), malachite green (66), thiouracil (67), 5-amino-7-hydroxy-V-triazolo (D) pyrimidine (68), and 2,4-dichlorophenoxyacetic acid (69) have all been described as reducing the multiplication of tobacco mosaic or certain other mechanically transmissible viruses. Exposing bean leaves to atmospheres containing 50 per cent carbon dioxide for the 2 hr. immediately before or after inoculation with tobacco necrosis viruses reduces the numbers of lesions (70) and immersing stem pieces infected with tobacco mosaic virus in solutions containing 1 mg. per l. naphthalene acetic acid (71) reduces the amount of extractable material that absorbs light at 260 $m\mu$. No inhibition of virus multiplication by antibiotics has yet been described, although several have been tested (57), but terramycin and streptothricin are stated (72) to prevent lesions on *Vigna sinensis* when inoculated with tobacco necrosis or tobacco ringspot viruses; the lesions develop on detached leaves in the dark only when the medium contains glucose. Their formation is also prevented by sodium azide and cyanide. When leaves are sprayed either

before or after inoculation with solutions containing 50 mg. per l. of tricothecin the numbers of lesions caused by tobacco necrosis and tobacco mosaic viruses are reduced (73). Many of these materials are phytotoxic and, in the absence of any information about their effects on the metabolism and subsequent development of treated plants, it may well be that they have no specific action against viruses but are causing a general dislocation of host metabolism.

No survey of factors that affect susceptibility would be complete that did not mention other infections. Most attention has been given to the phenomenon of interference between related strains of a virus and the protection afforded by systemic infection with one strain against further invasion by others. The literature on this subject is too large to review here, but it may be summarised by saying that the presence of one strain seems not to prevent the entry of another but to prevent it from multiplying enough to produce its normal effects. In general a virus protects a plant from subsequent attack by another virus to which the first is serologically related and, with potato virus X, the protection afforded is correlated with the closeness of serological relationship (74). Tobacco veinal necrosis and potato virus Y are exceptions to this generalisation, for although closely related serologically, they do not protect plants against one another (75). When plants are infected simultaneously with two unrelated viruses, symptoms are often more severe or of a different type from those caused by either alone. It seems that this may be correlated with increased multiplication of one of the viruses. For example, sap from potato and tobacco plants infected simultaneously with potato viruses X and Y contains more virus X than sap from plants infected with this virus alone (76). Similarly, many species of plants infected with dodder latent mosaic virus first show symptoms and then recover, the recovery being accompanied by a great reduction in the infectivity of sap; if such recovered plants are infected with tobacco etch or tobacco mosaic viruses, they again develop the initial symptoms, and the concentration of dodder latent mosaic virus in sap is increased and maintained at a high level (77). One example has been reported of a fungal infection increasing susceptibility to a virus; the size of local lesions produced by tobacco mosaic virus in French bean leaves is much increased by infection with *Uromyces phaseoli* and the virus multiplies in fungus-infected tissues without causing the customary necrosis (78). The suppression of potato virus Y by severe etch virus has already been mentioned (40).

THE INFLUENCE OF INFECTION ON HOST METABOLISM

The great variety of symptoms evoked by infection with viruses demonstrates the many changes in metabolism that can be caused. Enough examples have already been given in this review to show that there is no invariable response produced by infecting a host of a given genotype with a particular virus; what happens can be determined entirely by the physiological condition of the host. No enzymes have been identified in preparations of plant

viruses that have been carefully purified, and the consequences of infection probably reflect the extent to which the normal enzyme systems of the host have been thrown out of balance. The literature contains many references to changed enzymic activities caused by infection. These have already been reviewed (79, 80) without any clear principles emerging. It seems fairly well established that infected plants respire more than uninfected ones, but even with tobacco mosaic virus in tobacco and tomato, the systems studied most, there is little agreement on details. Many of the apparent discrepancies may well result from the unwitting use of host plants in widely different physiological states. The only comparison that has been made of healthy and tobacco mosaic virus infected tobacco plants, grown with a wide range of fertilizer treatments, has shown (81) that infection did not produce changes in the protease and pectase content falling significantly outside the differences caused by the differences in fertilizer treatment.

There seems little doubt that, on the basis of changes in the carbohydrate/nitrogen ratios, diseases divide into two types. In plants with the yellows type of disease, the ratio is increased, at least when plants are adequately illuminated, whereas in plants with the mosaic type of disease, the ratio is reduced. Since it was first shown that phloem of potato plants with leaf roll becomes necrotic (82), phloem necrosis has been identified as a feature of many yellows diseases, and it has been assumed that carbohydrates accumulate in the leaves because they cannot be translocated through the degenerate sieve tubes. This view was early contested (83), but has continued to be held largely because when diseased and healthy plants are kept in the dark, the leaves of the former still stain strongly with iodine while those of the healthy plants do not. The view could be held only by neglecting the fact that mosaic viruses, which cause no phloem necrosis, also reduce the rate at which starch is lost from infected cells in darkness, for this fact is the basis of one technique for demonstrating local infections with viruses that normally produce no visible local lesions. Two methods can be used (84): the inoculated leaves can be decolourised and stained with iodine in the evening, when the infected areas show as light spots against a uniform background; or they may be stained after the plants have been in darkness for some hours, when the lesions show as dark areas against a light background. Thus, infection with the mosaic viruses reduces the rate at which starch is synthesised in daylight and broken down in darkness. There is no critical information on the way in which mosaic and yellows type viruses affect photosynthesis, but their different effects on carbon/nitrogen ratios may lie here rather than in effects on the phloem. That phloem necrosis is not responsible for the retention of starch in leaves of sugar beet plants with yellows has been shown by demonstrating that such leaves lose as much total carbohydrate during a period of darkness as leaves of uninfected plants (85). Older leaves of infected plants contain more total carbohydrate, both at the start and end of the dark period, than comparable leaves of healthy plants, but by far the greater part of the increase is in glucose and fructose, which

may amount to 20 per cent of the total dry weight—more than five times as much as in healthy leaves. In contrast to the effects of yellows virus, the carbohydrate content of beet leaves is little affected by infection with beet mosaic virus.

The position is less clear with the mosaic-type diseases. Most work has been done with plants suffering from tobacco mosaic. These contain larger quantities of anomalous nucleoprotein than plants infected with any other virus that has been studied. In this infection, therefore, the anomalous material is itself an analytically significant component of the leaf. Detectable changes in the chemical composition of the leaf occur, therefore, both because of the presence of virus and because of its effects on the physiological activities of the leaf. It is often asserted that, despite the large amount of anomalous protein in the infected leaf, the nitrogen content is not significantly affected by infection and that virus multiplication occurs at the expense of normal protein. Under extreme conditions this is obviously so, for virus will increase in a detached leaf, where, as is well known, proteolysis occurs, and there is no external source of nitrogen. When plants are adequately fertilised, however, systemic infection with tobacco mosaic virus leads to an increase in the total nitrogen per plant and also in the percentage of nitrogen in the dry matter of the plant (81). It is probable therefore that the similarity in composition of normal and infected plants, when grown with inadequate nutrients as is not uncommon in normal glasshouse practice, is a coincidence.

Anomalous nucleoprotein can make up more than 10 per cent of the dry matter of leaves infected with tobacco mosaic virus, if the soluble and insoluble nucleoprotein is taken into account (11), but the actual amount is affected by the state of growth and nutrition of the plants (81). Thus there is a larger total in plants well supplied with nitrogen, but it amounts to a smaller proportion of the whole plant protein; in nitrogen-deficient plants it can account for 60 per cent of the total nitrogen and for 80 per cent of the nitrogen that is left attached to the leaf fibre after normal mincing.

A beginning has been made in the study of the changes in the soluble leaf proteins that follow infection with tobacco mosaic virus. For analytical convenience attention has been confined to that small proportion of the soluble protein that stays soluble in spite of freezing, ultracentrifugation, drying, and exposure to the concentrated components of sap (86). When studied electrophoretically there is one predominant component in this residual protein when uninfected tobacco leaves are used, but during infection the amount of this diminishes and a component with the characteristic electrophoretic mobility of tobacco mosaic virus preparations appears. Wildman, Cheo & Bonner (86) have used this as evidence that "virus protein is synthesised at the direct expense of the normal protein." Pirie (87) has argued that this conclusion is fallacious. He regards the normal protein as an artefact derived by the breakdown of a nucleoprotein that is readily ultracentrifuged from the sap of uninfected young tobacco plants. This nor-

mal nucleoprotein, however, is greatly diminished as the leaf matures normally and it is also diminished by virus infection. One of the obvious physiological results of virus infection is an increased rate of maturation of the leaf; it is this maturity, even before a definite cachectic state has been established, that leads to the disappearance of the normal nucleoprotein. If a protein is undergoing proteolysis in a leaf in which virus is being synthesised, it is reasonable to assume that some of the amino acids of the one protein will reappear in the other, in much the same way that the salmon's gonads are formed at the expense of its muscles. But such an interconversion, if it goes through the general amino acid pool of the organism, hardly comes into the category of synthesis at the "direct expense" of a normal protein. All that has been clearly established is that the concentration of certain proteins in the sap of infected plants is smaller than in normal plants of the same age (86, 87), but whether this is a direct effect of virus infection or an indirect effect that could have been achieved by any other means that increased the physiological age of the leaf is a matter for further experiment. It would, for example, be interesting to follow the changes in normal protein that accompany infection with viruses that produce very much smaller amounts of anomalous nucleoprotein.

Changes in the concentrations of low molecular weight components have not been followed directly during virus infection, but indirect evidence about the equilibria that are set up during virus synthesis has come from experiments with infected leaves infiltrated with ammonium chloride labelled with N^{15} (88). This leads to the conclusion that tobacco mosaic virus is formed from compounds such as amino acids that more readily exchange nitrogen with ammonium ion than do formed proteins, and, also, that its formation is an irreversible process, with the virus not in equilibrium with other cell proteins. Although this second conclusion may be largely true of such a stable virus as tobacco mosaic, which accumulates in large amounts, there is no reason to expect it also to be true of other viruses that are less stable *in vitro* and that early reach a low maximum and then remain constant or fall to much lower levels.

Most work on the protein content of infected plants has been done in the hope of isolating something that could be identified as the virus itself. The emphasis has been on the homogeneity of the end product and little attention has been given to material that was not infective. This has tended to encourage the view that virus infection consists of the replication of an initial infecting unit, with a uniform end product. There is, however, considerable evidence that this picture is false and that infection can lead to the production of a variety of end products, only some of which are able to initiate infection in new hosts. Anomalous nucleoproteins of different particle sizes and infectivities occur in extracts of plants infected with tobacco mosaic virus (89) and the Rothamsted culture of tobacco necrosis virus (90, 91). Particles of similar sizes, but not all of which are nucleoprotein, occur in sap from plants infected with turnip yellow mosaic virus (92). There is evi-

dence from serology and electron microscopy that plants infected with sugar beet yellows (93) and broad bean mottle (94) viruses may also contain more than one type of anomalous particle; a lipoprotein that has not been recorded in extracts of healthy plants was noted in extracts from plants infected with potato virus Y (95). The significance and origins of these various products are unknown, but they warrant further study, for they may well be important *in vivo* even though they cannot all initiate infection.

One kind of change induced by infection warrants mention because of its possible implications in affecting the spread of virus diseases. Leaf-hoppers of several species have been found to breed more freely, pass through nymphal stages sooner, and live longer, when feeding on celery and aster plants infected with aster yellows virus than on healthy plants (96); similarly *Aphis fabae* has been found to thrive better on beet plants with mosaic than on healthy plants (97).

The physiological bases for the production of the symptoms by which viruses are customarily recognised, and to which they owe their economic importance, remain almost wholly unknown. It is unlikely that they are the result of a simple sequestration of protein or nucleic acid precursors. If this were so, severity of symptoms might be expected to be directly correlated with the amounts of anomalous protein extractable from systemically infected plants, but it is not. Tobacco mosaic is a mild disease compared with others caused by many viruses that achieve only a thousandth or less of its concentration, and the symptoms caused by different strains of tobacco mosaic (98), potato X (99, 100) or potato Y (75) viruses, are no sure guide to the extents to which they occur in diseased plants. The fact, too, that symptoms may be strikingly altered by altering the physiological condition of the host indicates that they are not a necessary consequence of virus multiplication, but side effects reflecting secondary changes in host-plant reactions.

Infected plants often show symptoms suggesting that the normal hormone balance is disturbed. Infection of tomato leaves with spotted wilt virus has been found to diminish their auxin content (101). Changes in the response of plants to applied hormones have also been described; sweet potato plants with witches' broom disease reacted less than normal plants when treated with β -indolylacetic acid (102), whereas plants infected with southern bean mosaic or aster yellows viruses reacted more severely than uninfected ones to β -naphthoxyacetic acid (103). The necrotic lesions that commonly result from virus infections fluoresce in ultraviolet light (104). The substance responsible has been identified as 6-methoxy-7-hydroxycoumarin (scopoletin) (105, 106). This occurs in small quantities in uninfected plants and Best (105) suggests that it is normally an intermediate product in a series of syntheses that is interrupted by infection. Until evidence is forthcoming that scopoletin kills cells, however, it remains uncertain whether its accumulation is the direct cause of necrosis, or *vice versa*. Necrotic lesions have also been described as producing unusually

large quantities of an epinasty-producing emanation, probably ethylene (107).

In summary, we suggest that virus multiplication is comparable neither with the growth of a bacterium in a culture medium nor with the direct conversion of a precursor into an enzyme. Rather, it is a metabolic disturbance, which, with the viruses so far studied in any detail, primarily involves nucleoprotein metabolism. Little is known about the mechanism of the normal metabolism, but it proceeds in leaves, for these contain deoxy-nucleoproteins in the chromosomes and pentose nucleic acids in the microsomes and other such bodies. The metabolism of deoxynucleoproteins may not be involved, because no plant virus has yet been found to contain deoxypentose. This may well be a consequence of the fact that those so far studied chemically, characteristically affect cells that have ceased to divide and in which chromosome metabolism, therefore, is probably minimal; it may not be true of such viruses as wound-tumour, which cause excessive proliferation in tissues that have normally ceased division (108). An extensive range of activities is generally attributed to the microsomes. If these are affected by virus multiplication there would be ample explanation for the diverse results of infection. If viruses do intrude into such systems, the help to virus research that would accrue from a better understanding of their physiological activities is too obvious to need stressing. It may be less obvious that virus research can give reciprocal help to Plant Physiology.

LITERATURE CITED

1. Black, L. M., *Nature*, **166**, 852 (1950)
2. Maramorosch, K., *Phytopathology*, **41**, 25 (1951)
3. Crafts, A. S., *Botan. Rev.*, **17**, 203 (1951)
4. Bald, J. G., *Ann. Applied Biol.*, **24**, 33 (1937)
5. Bald, J. G., *Ann. Applied Biol.*, **24**, 56 (1937)
6. Bald, J. G., *Australian J. Exptl. Biol. Med. Sci.*, **15**, 211 (1937)
7. Youden, W. J., Beale, H. P., and Guthrie, J. D., *Contribs. Boyce Thompson Inst.*, **7**, 37 (1935)
8. Lauffer, M. A., and Price, W. C., *Arch. Biochem.*, **8**, 449 (1945)
9. Kleczkowski, A., *J. Gen. Microbiol.*, **4**, 53 (1950)
10. Kleczkowski, A., and Kleczkowski, J., *J. Gen. Microbiol.*, **5**, 346 (1951)
11. Bawden, F. C., and Pirie, N. W., *Brit. J. Exptl. Path.*, **27**, 81 (1946)
12. Bhargava, K. S., *Ann. Applied Biol.*, **38**, 377 (1951)
13. Bawden, F. C., and Pirie, N. W., *Brit. J. Exptl. Path.*, **25**, 68 (1944)
14. Baur, E., *Sitzber. kgl. preuss. Akad. Wiss.*, **1**, 11 (1906)
15. Ainsworth, G. C., *Ann. Applied Biol.*, **22**, 55 (1935)
16. Knight, C. A., *Arch. ges. Virusforsch.*, **2**, 26 (1941)
17. Welsh, F. M., and Wilks, J. M., *Phytopathology*, **41**, 136 (1951)
18. Bawden, F. C., and Roberts, F. M., *Ann. Applied Biol.*, **34**, 286 (1947)
19. Bawden, F. C., and Roberts, F. M., *Ann. Applied Biol.*, **35**, 418 (1948)
20. Bawden, F. C., *Rothamsted Exptl. Sta. Ann. Rept.* (1950)
21. Hougas, R. W., *Phytopathology*, **41**, 483 (1951)
22. Bawden, F. C., and Kleczkowski, A. (Unpublished data)
23. Larsen, P., *Ann. Rev. Plant Physiol.*, **2**, 169 (1951)
24. Tolbert, N. E., and Burris, R. H., *J. Biol. Chem.*, **186**, 791 (1950)
25. Best, R. J., *Australian J. Exptl. Biol. Med. Sci.*, **14**, 223 (1936)
26. Johnson, J., *Phytopathology*, **12**, 438 (1922)
27. Bennett, C. W., *Mich. State College Agric. Exptl. Sta. Tech. Bull.*, No. 80 (1927)
28. Holmes, F. O., *Phytopathology*, **24**, 845 (1934)
29. Pound, G. S., and Walker, J. C., *J. Agr. Research*, **71**, 255 (1945)
30. Pound, G. S., and Walker, J. C., *J. Agr. Research*, **71**, 471 (1945)
31. Samuel, G. S., *Ann. Applied Biol.*, **18**, 494 (1931)
32. Kunkel, L. O., *Phytopathology*, **26**, 809 (1936)
33. Kunkel, L. O., *Am. J. Botany*, **23**, 683 (1936)
34. Hutchins, L. M., and La Rue, J. L., *Phytopathology*, **29**, 12 (1939)
35. Hildebrand, E. M., *Contribs. Boyce Thompson Inst.*, **11**, 485 (1941)
36. Kunkel, L. O., *Am. J. Botany*, **28**, 761 (1941)
37. Kunkel, L. O., *Proc. Am. Phil. Soc.*, **86**, 470 (1943)
38. Kunkel, L. O., *Phytopathology*, **35**, 805 (1945)
39. Kassanis, B., *Ann. Applied Biol.*, **37**, 339 (1950)
40. Bawden, F. C., and Kassanis, B., *Ann. Applied Biol.*, **32**, 52, (1945)
41. Kassanis, B. (Unpublished data)
42. Spencer, E. L., *Phytopathology*, **25**, 178 (1935)
43. Spencer, E. L., *Phytopathology*, **25**, 493 (1935)
44. Bawden, F. C., and Kassanis, B., *Ann. Applied Biol.*, **37**, 46 (1950)
45. Spencer, E. L., *Plant Physiol.*, **14**, 769 (1939)
46. Spencer, E. L., *Plant Physiol.*, **16**, 227 (1941)
47. Spencer, E. L., *Plant Physiol.*, **16**, 663 (1941)

48. Spencer, E. L., *Science*, **94**, 96 (1941)
49. Spencer, E. L., *Plant Physiol.*, **17**, 210 (1942)
50. Bawden, F. C., and Kassanis, B., *Ann. Applied Biol.*, **37**, 215 (1949)
51. Chessin, M., *Phytopathology*, **41**, 235 (1951)
52. Miller, G. L., and Stanley, W. M., *J. Biol. Chem.*, **146**, 331 (1942)
53. Felton, M. W., *Am. Potato J.*, **25**, 50 (1948)
54. Brierley, P., and Stuart, N. W., *Phytopathology*, **36**, 297 (1946)
55. Bawden, F. C., and Pirie, N. W., *Biochem. J.*, **37**, 70 (1943)
56. Tinsley, T. W., *The Effects of Variations in Water Supply on the Susceptibility of Plants to Virus Diseases* (Doctoral thesis, London Univ., London, England, 1951)
57. Weindling, R., Katznelson, H., and Purdy Beale, H., *Ann. Rev. Microbiology*, **4**, 247 (1950)
58. Brierley, P., and Smith, F. F., *Plant Disease Repr.*, **34**, 363 (1950)
59. Van der Want, J. P. H., *Tijdschr. Plantenziekten*, **57**, 72 (1951)
60. Loring, H. S., *J. Gen. Physiol.*, **25**, 553 (1942)
61. Stoddard, E. M., *Phytopathology*, **32**, 17 (1942)
62. Stoddard, E. M., *Connecticut Agr. Exptl. Sta. Bull.*, No. 506 (1946)
63. Rumley, G. E., and Thomas, W. D., *Phytopathology*, **41**, 301 (1951)
64. Woods, M. W., *Phytopathology*, **33**, 77 (1943)
65. Rischkov, V. L., Smirnova, V. A., and Gorodskaja, O. S., *Biokhimiya*, **11**, 197 (1946)
66. Takahashi, W. N., *Science*, **107**, 226 (1948)
67. Commoner, B., and Mercer, F., *Nature*, **168**, 113 (1951)
68. Matthews, R. E. F., *Nature*, **167**, 892 (1951)
69. Limasset, P., Levieil, F., and Sechet, M., *Compt. rend.*, **227**, 643 (1948)
70. Kalmus, H., and Kassanis, B., *Nature*, **154**, 641 (1944)
71. Kutsky, R. J., and Rawlins, T. E., *J. Bact.*, **60**, 763 (1950)
72. Leben, C., and Fulton, R. W., *Phytopathology*, **41**, 23 (1951)
73. Bawden, F. C., and Freeman, G. G. (Unpublished data)
74. Matthews, R. E. F., *Ann. Applied Biol.*, **36**, 460 (1949)
75. Bawden, F. C., and Kassanis, B., *Ann. Applied Biol.*, **32**, 402 (1951)
76. Ross, A. F., *Phytopathology*, **40**, 24 (1950)
77. Bennett, C. W., *Phytopathology*, **39**, 637 (1949)
78. Yarwood, C. E., *Phytopathology*, **41**, 39 (1951)
79. Wynd, F. L., *Botan. Rev.*, **9**, 395 (1943)
80. Bawden, F. C., *Plant Viruses and Virus Diseases* (Chronica Botanica Co., Waltham, Mass., 335 pp., 1950)
81. Holden, M., and Tracey, M. V., *Biochem. J.*, **43**, 151 (1948)
82. Quanjer, H. M., *Mededeel Rijks Hoog. Land- Tuin-Boschbouwschool Wageningen*, **6**, 41 (1913)
83. Murphy, P. A., *Sci. Proc. Roy. Dublin Soc.*, **17**, 163 (1923)
84. Holmes, F. O., *Contribs. Boyce Thompson Inst.*, **14**, 457 (1931)
85. Watson, M. A., and Watson, D. J., *Ann. Applied Biol.*, **38**, 276 (1951)
86. Wildman, S. G., Cheo, C. C., and Bonner, J., *J. Biol. Chem.*, **180**, 985 (1949)
87. Pirie, N. W., *Biochem. J.*, **47**, 614 (1951)
88. Meneghini, M., and Delwiche, C. C., *J. Biol. Chem.*, **180**, 177 (1951)
89. Bawden, F. C., and Pirie, N. W., *Brit. J. Exptl. Path.*, **26**, 294 (1945)
90. Bawden, F. C., and Pirie, N. W., *Brit. J. Exptl. Path.*, **26**, 277 (1945)

91. Bawden, F. C., and Pirie, N. W., *J. Gen. Microbiol.*, **4**, 464 (1950)
92. Markham, R., and Smith, K. M., *Parasitology*, **39**, 330 (1949)
93. Nixon, H. L., and Watson, M. A., *Nature*, **168**, 523 (1951)
94. Bawden, F. C., Chaudhuri, R. P., and Kassanis, B., *Ann. Applied Biol.*, **38**, 774 (1951)
95. Bawden, F. C., and Pirie, N. W., *Brit. J. Exptl. Path.*, **20**, 322 (1939)
96. Severin, H. H. P., *Hilgardia*, **17**, 121 (1946)
97. Kennedy, J. S., *Nature*, **168**, 824 (1951)
98. Bawden, F. C., and Pirie, N. W., *Proc. Roy. Soc. (London)*, [B] **123**, 274 (1937)
99. Bawden, F. C., and Pirie, N. W., *Brit. J. Exptl. Path.*, **19**, 66 (1938)
100. Bawden, F. C., and Crook, E. M., *Brit. J. Exptl. Path.*, **28**, 403 (1947)
101. Grieve, B. J., *Australian J. Exptl. Biol. Med. Sci.*, **21**, 89 (1943)
102. Thung, T. H., and Hadiwidjaja, T., *Tijdschr. Plantenziekten.*, **57**, 95 (1951)
103. Hartman, R. T., and Price, W. C., *Am. J. Botany*, **37**, 820 (1950)
104. Best, R. J., *Australian J. Exptl. Biol. Med. Sci.*, **14**, 199 (1936)
105. Best, R. J., *Australian J. Exptl. Biol. Med. Sci.*, **22**, 251 (1944)
106. Andreae, W. A., *Can. J. Research*, [C] **26**, 31 (1948)
107. Ross, A. F., and Williamson, C. E., *Phytopathology*, **41**, 431 (1951)
108. Black, L. M., *6th Symposium on Development and Growth*, 79 (1947)

MECHANISMS OF ION ABSORPTION BY ROOTS¹

BY ROY OVERSTREET AND LOUIS JACOBSON

*Division of Soils and Division of Plant Nutrition, University of California,
Berkeley, California*

INTRODUCTION

In view of the recent comprehensive reviews by Robertson (13) and by Hewitt (4) and because of the fact that detailed annual coverage of the subject is readily available in abstract form, it was felt desirable to confine this article largely to the basic feature of mineral nutrition, namely, ion absorption by roots.

Understandably, over the past 30 years there has been a growing interest in the process of mineral absorption by plant cells. From the point of view of practical agriculture, the absorption reactions are intimately tied up with nutritional and toxicity problems in connection with the growing of crops in soil. Also, since the process frequently involves the accumulation of elements in cells against gradients in chemical potential, the phenomenon of ion absorption is of considerable theoretical interest. Just as photosynthesis in plants has aroused the curiosity of physiologists and chemists in that it represents a transformation of radiant energy into chemical energy, so also is ion absorption of interest because apparently it involves a transformation of chemical energy into osmotic energy.

As the study of mineral uptake by plant and animal cells has progressed over recent decades, theories and mechanisms designed to explain the process have been proposed in abundance. These mechanisms fall roughly into two general classes; namely, (a) those which interpret the process in terms of special physical or electrical characteristics of cellular membranes, and (b) those which postulate regions with special chemical properties. In general, it can be said that the average life of these theories has been short because of the continual uncovering of new experimental evidence which has placed fresh burdens on or completely eliminated existing theories.

As matters stand at present, no proposed mechanism for ion absorption is universally accepted. Nevertheless, the salient features of the process have been reasonably well established for a variety of plant tissues. Thus we are at least in a favorable position to weigh and evaluate theories as they are proposed. More specifically, any theory of ion uptake by roots must be consistent with the following uniformly observed facts concerning the process:

(a) The ion absorption process requires metabolic activity by the plant (isotopic exchanges excepted). No ion accumulation occurs in the absence of respiration and other activities such as protein synthesis, etc. In general, the process is an attribute of tissues capable of growth although not invari-

¹ The survey of the literature pertaining to this review was concluded in November, 1951.

ably so. When the metabolic activity of the plant is inhibited by reduced oxygen tension, lowered temperature, or metabolic poisons, ion accumulation is likewise inhibited. This is true for the accumulation of both anions and cations.

(b) The ion absorption process is an exchange process. Cations are usually absorbed in exchange for H^+ ions of the plant which are released to the culture medium. For every anion absorbed, an OH^- or HCO_3^- ion appears in the culture medium. The evidence indicates that no ion passes in or out of a normal plant root except by exchange for another ion. Moreover, isotopic exchanges between accumulated isotopes and those of the culture medium have been observed for a variety of elements. This type of exchange may occur in the absence of metabolic activity by the plant.

(c) Ion accumulation is to a large extent selective. As a result of the exchange character of the process, anions and cations can enter the plant individually. Also, ions are not absorbed at the same rates. In general, the cations K^+ , NH_4^+ , Rb^+ , and Cs^+ are rapidly accumulated, while Ca^{++} , Mg^{++} , and Ba^{++} are much more slowly taken up. The anions NO_3^- , Br^- , and Cl^- are usually rapidly absorbed. The anions SO_4^{--} and $H_2PO_4^-$ are rather slow-moving; there is some evidence that the anion HCO_3^- may not be absorbed at all.

(d) Marked influences of one ion of the culture medium upon another in absorption have been observed. For ions of the same charge, this influence is usually of a competitive nature. On the other hand, the presence of ions such as Ca^{++} , Sr^{++} , Ba^{++} , Al^{+++} , and Fe^{+++} has been observed to exert a stimulating effect on the absorption of K^+ , Rb^+ , Cs^+ , and Br^- under some conditions. Further, a slowly absorbed cation or anion exerts a depressing effect on the absorption of its associated ion.

NATURE OF THE ABSORPTION PROCESS

In recent years there has been an increasing tendency among both plant and animal physiologists to favor a chemical rather than a physical or electrical explanation of ion accumulation. This is the result of considerations such as are embodied in the studies of Spiegelman & Reiner (17). Because of its bearing on absorption by roots, their kinetic analysis of potassium accumulation and sodium exclusion by animal tissues such as frog's muscle deserves consideration at this point.

The treatment of Spiegelman & Reiner is based on the assumption that the transport of ions or molecules within cells is governed by the customary laws of kinetic theory; that is, that the distribution function of velocity for ions within a uniform phase (in the absence of external forces of a directional kind) is given by the Maxwell-Boltzman distribution function of velocity for molecules in a perfect gas. Their development of a general theory of transport consists in a study of effects on the distribution of the ions caused by the imposition of external forces, such as electric fields, or of internal inequalities, such as mobility differences, or of the introduction of phase boundaries. Proceeding on this basis, Spiegelman & Reiner were led to the follow-

ing interesting conclusions concerning the problem of potassium and sodium balance in some animal tissues:

(a) Mobility differences cannot yield a mechanism for selection of one ion in preference to another by systems in the steady state [Teorell (19) systems].

(b) Electrostatic barriers similarly cannot provide a selective mechanism (i.e., Donnan systems).

(c) Selective membrane theories in general make implicit assumptions about potential distribution in the cytoplasm as compared with the membrane which do not seem plausible and in any case would require separate justification.

(d) On the basis of data for frog muscle suspended in Ringer's solution, calculations are made which indicate that to concentrate potassium in the presence of sodium to the extent found in such tissue requires an energy supply of the order of 10,000 cal. per mole.

(e) Physical forces such as those of polarization, or interaction of polar groups in membranes, and London type dispersion forces which might conceivably be used by membrane theories are inadequate.

(f) On the basis of the energy requirements, chemical as distinguished from electrical forces would provide a more fruitful approach to the problems of potassium and sodium balance.

The accumulation process in plant cells, that is, the transfer of chemical matter from a lower to a higher chemical potential, is now quite generally assumed by plant physiologists to take place by means of a coupling to intracellular chemical processes. However, the mechanism of such couplings or the manner in which a chemical reaction is able to force upon a chemical substance a direction of movement opposite to its natural diffusion tendency are at the present time largely matters of pure speculation.

ION ABSORPTION MODELS

In a recent paper, Rosenberg (16) has attempted the elucidation of the energetic mechanism in processes of this kind by means of a thermodynamic treatment of certain basic model systems. In the authors' opinion, his analysis contributes greatly to the clarification of the problem and warrants consideration here in its essential features.

As his model system Rosenberg assumes that the chemical substances A, B, etc., are localized in two phases (i) and (o) of given compositions which do not vary in time. Also, homogeneity with respect to temperature and pressure is assumed. Further, a connecting link, M, is assumed through which the possibility of communication of A, B, etc., between the two phases is maintained. The object of Rosenberg's treatment is to examine the conditions associated with the transport of the infinitesimal quantities dn_A , dn_B , etc., through M. The properties of the connecting link, M, are defined for each individual system considered. Of particular interest here are the systems corresponding to what Rosenberg has termed "transport in partial equilibrium" and "transport in stationary systems."

Transport in partial equilibrium.—For the illustration of this mode of

transport, the two phases (i) and (o) of the model system (Figure 1) are assumed to contain the two substances A and B. In addition, it is assumed that the connecting link M is impermeable to the solvent and to A and B but permeable to a compound (AB) of the two substances. By means of thermody-

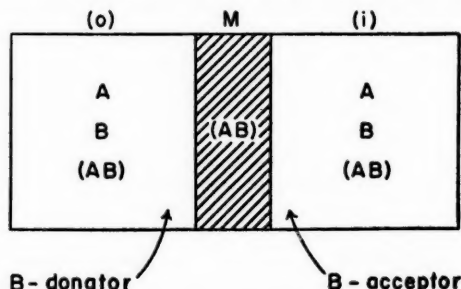


FIG. 1. Rosenberg's model for transport in partial equilibrium. The solutes A, B, and¹(AB) are localized in the solutions (o) and (i). The connecting link M is permeable to the substance (AB) but is impermeable to the solvent and the substances A and B. The presence of a B-donator in (o) and a B-acceptor in (i) permits a transport of A from (o) to (i) against a gradient in chemical potential.

namic reasoning it can be shown that a type of equilibrium is possible in such a system where $\mu_{A(i)} > \mu_{A(o)}$ and $\mu_{B(o)} > \mu_{B(i)}$, the symbol μ denoting the chemical potential.² The conditions of equilibrium are that μ_{AB} is constant throughout the whole system and

$$\mu_{A(i)} - \mu_{A(o)} = \mu_{B(o)} - \mu_{B(i)}. \quad 1.$$

Regarding the above equilibrium, Rosenberg points out that

If M is selectively permeable to AB, an infinitesimal transport of the complex in one direction will cause an infinitesimal tendency towards transport in the opposite direction at equilibrium. A one-sided transport of major quantities, therefore, cannot take place without cooperation with the surroundings.

He goes on to say:

If the phase (o) is connected to a B-donator and the phase (i) to a B-acceptor, finite quantities of the complex can be transported from (o) to (i), this transport resulting in an accumulation of A in (i) opposite to its chemical potential gradient. This may, for example, take place if the two phases are in communication with two B-reservoirs in the surroundings. In biological systems the most frequent case is that a certain accumulation is supposed to be associated with a chemical reaction in phase (i). It is obvious that such a coupling can be established in the present case if this reaction works as a B-acceptor, i.e., if B is consumed during the reaction. Suppose that in phase (i) B reacts with a non-diffusible substance C, which results in the formation of the compound BC, and that this reaction takes place in equilibrium. In this way the

² In this model, and in all others treated in this paper, it is assumed that the inside and outside phases are at the same electric potential.

coupling with a chemical reaction sought for is established. Besides equation 1 the relation:

$$\mu_{B(i)} + \mu_{C(i)} = \mu_{BC(i)} \quad 2.$$

holds in this system. By combining 2 with 1 we obtain:

$$\mu_{A(i)} - \mu_{A(o)} = \mu_{B(o)} + \mu_{C(i)} - \mu_{BC(i)}. \quad 3.$$

From 3 it appears that the chemical energy necessary for the active transport of A originates from the chemical reaction



in which neither the complex AB nor the substance A in any form are included.

Rosenberg concludes further: "Moreover it is seen from 3 that the energy transformation is complete, the energetic efficiency being 100%. This condition holds in general for systems in partial equilibrium."

It appears that the latter statement of Rosenberg concerning the energetic efficiency is in error. The relationships of equations 2 and 3 are valid for the system in equilibrium. However, for a finite transport of A from (o) to (i), the reaction $B_{(o)} + C_{(i)} \rightarrow BC_{(i)}$ must take place spontaneously, that is, with a decrease in free energy or must be coupled to some other reaction that takes place spontaneously. Also, the extent of the transport will depend on the amount of $BC_{(i)}$ formed. However, in general the amount of $BC_{(i)}$ formed will bear no relationship to the free energy change in the spontaneous process. For this reason, calculations of the energetic efficiency of the transport process in such a model are meaningless. The general question of the efficiency of accumulation processes will be discussed more fully in a later section.

It should be noted that the function of the substance B in the system just described may be taken as a carrier function, the active transport of A being induced by B's positive transport tendency in the total transport. Also, any specificity in the accumulation of A as compared to that of other substances present presumably must be ascribed to the specific nature of the complex AB.

Transport in stationary systems.—As emphasized in the foregoing discussion, the maintenance of a state of partial equilibrium and the process of transport in partial equilibrium is associated with very stringent requirements on the permeability of the connecting link M; that is, M is permeable to the compound AB but not to the solvent and to the substances A and B. Rosenberg points out that a relaxation of these requirements, so that the connecting link is also permeable to either A or B, may result in a state of "stationary equilibrium." Associated with this type of equilibrium, is the possibility of "transport in stationary systems." Moreover, it follows from the theory that when this mode of transport is coupled to a chemical reaction, the "efficiency" is always less than in the case of transport in partial equilibrium in that a greater donation and acceptance of the carrier substance B is required to effect the transport of a given amount of the solute A.

To illustrate, we shall assume that the phases (o) and (i) are two homogeneous solutions of A and B in the same solvent. Also we shall assume that potential gradients are maintained in the connecting link by a supply from outside of B to (o) and removal of B from (i). With these assumptions we shall consider two simplified limiting cases concerning the permeability of the connecting link M:

(a) M is permeable to AB and B but not to A. Since A is transported through M exclusively in the form of AB in this system, AB is in equilibrium in the whole system, and, as with the system in partial equilibrium,

$$\mu_{A(i)} - \mu_{A(o)} = \mu_{B(o)} - \mu_{B(i)}.$$

However, unlike the system in partial equilibrium, this state must be stabilized by B's irreversible diffusion through the system. Thus a constant quantity of B passes through each section of the system in unit time. For the maintenance of given potential differences this quantity will be the smaller, the smaller the concentration gradient of B in the connecting link. This means that the "efficiency" increases with decreasing permeability of the substance B in M. If an active transport of A is accomplished by increased supply of B from outside to (o), B will flow through M partly in the form of B as such and partly in the form of AB. Hence, it appears that the efficiency increases with increasing solubility and rate of diffusion of AB in M.

(b) M is permeable to AB and A but not to B. In this system B is transported through M exclusively in the form of AB so that

$$\mu_{AB(o)} > \mu_{AB(i)}.^3$$

$$\mu_{A(i)} - \mu_{A(o)} < \mu_{B(o)} - \mu_{B(i)}.$$

In the stationary state a constant amount of AB passes through each section of M in the direction (o) to (i), and a corresponding amount of A passes in the opposite direction in unit time. Moreover, this amount is equal to the amount of B supplied to (o) and removed from (i). Analogous to 1 a low permeability of A and a high permeability of AB in the connecting link will tend to increase the "efficiency" of transport of A.

The "osmotic diffusion pump" proposed earlier by Franck & Mayer (3) corresponds to a case of transport in stationary systems in Rosenberg's terminology. The connecting link of the diffusion pump consists of a solution between two membranes (see Figure 2). Franck & Mayer assume that the connecting link contains two solutes, a and b, and that b is an n-fold polymer of a. The authors assume further that:

- (a) D_a , the diffusion constant of a is less than n-fold, greater than D_b , the diffusion constant of b. This means that the same amount of material can diffuse more readily in the b form than in the dissociated form a.

³ This is so since A must be transported continuously from (o) to (i) to maintain its gradient. Since species A actually moves in the opposite direction, AB must flow from (o) to (i) at a greater rate. This demands that $\mu_{AB(o)} > \mu_{AB(i)}$ be maintained.

- (b) The conversion of na to b and vice versa is inhibited within the connecting link.
- (c) The conversion $na \rightarrow b$ is catalyzed to equilibrium at the membrane at $x=1$.
- (d) The supply of some other material at the membrane at $x=0$ dissociates b to na , in excess of the normal equilibrium, by some chemical change in the supplied material which releases free energy.

On the basis of the foregoing assumptions, Franck & Mayer conclude that

there will be a constant cycling of the solutes a and b . The dissociated form a , produced at the wall at $x=0$, will exist there at high concentration. It will diffuse to the

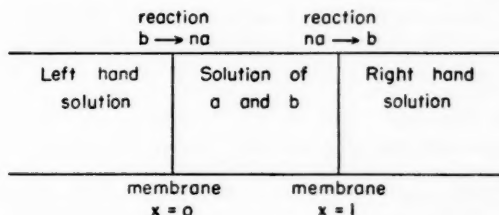


FIG. 2. Diagram of the osmotic diffusion pump proposed by Franck and Mayer. The connecting link here is a solution of the solutes a and b . This solution is bounded by membranes at $x=0$ and $x=1$. The solute b is an n -fold polymer of a . The conversion $na \rightarrow b$ is catalyzed at the membrane at $x=1$, and b is dissociated to na at the membrane at $x=0$. For the case where the membranes are permeable to a but not to b , the solute a will accumulate in the left-hand solution at a higher chemical potential than in the right-hand solution.

wall at $x=1$, where it is at a lower concentration, and be converted here, catalytically, to the associated form b , which has then higher concentration at $x=1$ than at $x=0$. The associated molecule b will diffuse from $x=1$ to the wall at $x=0$, where it is reconverted to the dissociated form a .

The authors conclude further that

Due to our assumptions about the relative diffusion constants, $D_a/nD_b = x < 1$, the gradient in concentration of b will be less than that of a , so the total concentration will be less on the right, at $x=1$, than on the left at $x=0$.

Franck & Mayer then proceed to consider a number of cases for which various assumptions are made concerning the permeability of the membranes. Of particular interest here is the case where the membranes are assumed to be permeable to a but not to b . In this case a dynamic equilibrium will be set up in which the chemical potential of the solute a will be higher in the left-hand solution than in the right-hand solution.

A supply of free energy to the connecting link is required to hold the dynamic equilibrium. If, however, the rate of the reaction supplying free energy is increased, a transfer of the solute *a* from the right-hand solution to the left-hand solution against a gradient in chemical potential is effected.

The foregoing is a statement of Franck & Mayer's theory in its simplest form. The authors point out that the formation of polymers is not essential to the theory. They speculate that in the case of ions, such as Na^+ and Cl^- , substances of types RH_n and $\text{R}'(\text{OH})_n$ may be produced in the cell at the membrane at $x=1$. These substances then react with the ions, as, for example, as follows:



The compound RNa_n then diffuses with a concentration gradient to the membrane at $x=0$, where the above reaction is reversed by the expenditure of free energy of some other reaction. If the membranes are assumed to be permeable to Na^+ , then that ion would accumulate in the left-hand solution by means of this process. It will be noted that such a transfer is very nearly equivalent to Rosenberg's "transport in stationary systems" for the case where the connecting link *M* is permeable to *AB* and *A* but not to *B*.

It is interesting to compare the theoretical models of cells proposed by Rosenberg and Franck & Mayer with an actual model constructed much earlier by Osterhout (10). On the basis of evidence that one or both of the protoplasmic surfaces in cells are characterized by a nonaqueous layer, Osterhout constructed a model of a cell in which the nonaqueous layer was represented by guaiacol. That is, in the model an aqueous solution, (o), representing the external medium, was separated from another aqueous solution, (i), representing the vacuolar sap, by a layer of guaiacol (HG). Carbon dioxide was continuously bubbled in the solution, (i), to imitate its production by the living cell.

When potassium as KCl was added to the solution (o), it was found to accumulate in the solution (i) as KHCO_3 . Presumably the model cell operated by means of the following reactions: In the solution (o), K^+ ion reacts with guaiacol to form potassium guaiacolate,



The potassium guaiacolate then moves with its diffusion gradient through the guaiacol layer to the solution (i), where potassium is released by means of the reaction,



Since the guaiacol layer is essentially impermeable to free ions such as K^+ and H^+ , K^+ accumulates in (i) as a result of the fact that the concentration of H^+ is maintained at a higher level in (i) than in (o).

Osterhout found that the guaiacol layer was more permeable to potassium guaiacolate than to sodium guaiacolate. Thus when KG and NaG were

added in equivalent amounts to (o), the resulting concentration of K^+ in (i) was about twice that of Na^+ .

It will be noted that although the accumulation of ions in the model depends on the existence of a connecting link that is impermeable to free ions but permeable to complexes of the ions (KG, NaG, etc.), Osterhout's cell has no exact counterpart in any of the cases considered by Rosenberg or Franck & Mayer.

Because of its possible importance in biological systems, the theory of Osterhout's model deserves consideration at this point. To this end we shall assume that the chemical substances A, B, (AX), and (BX) are localized in two phases (i) and (o) at constant temperature and pressure. Also we shall

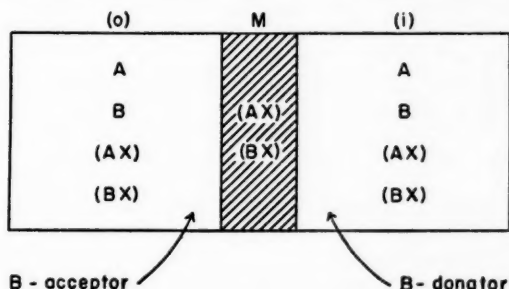


FIG. 3. A model for transport in partial equilibrium developed from Osterhout's experiments. The solutes A, B, (AX), and (BX) are localized in the solutions (o) and (i) under the conditions of the equilibrium: $A + (BX) \rightleftharpoons (AX) + B$. The connecting link M is permeable to the substances (AX) and (BX) but is impermeable to the solvent and the substances A and B. The presence of a B-donator in (i) and a B-acceptor in (o) will result in the transport of A from (o) to (i) against a gradient in chemical potential.

assume a connecting link M between (i) and (o), which is impermeable to the solvent and to A and B but permeable to the substances (AX) and (BX) (Figure 3). Further we shall assume that the following equilibrium obtains in (i) and (o):



On the basis of the foregoing assumptions, it can be shown thermodynamically that a partial equilibrium is possible in this model where $\mu_{A(i)} > \mu_{A(o)}$ and $\mu_{B(i)} > \mu_{B(o)}$. The conditions of equilibrium are that $\mu_{(AX)}$ and $\mu_{(BX)}$ are constant throughout the whole system and

$$\mu_{A(i)} - \mu_{B(i)} = \mu_{A(o)} - \mu_{B(o)}.$$

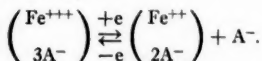
If in this model phase (i) is connected to a B-donator and phase (o) to a B-acceptor, finite quantities of A can be transported from (o) to (i) against a gradient in chemical potential.

It should be noted on comparing Osterhout's and Rosenberg's models for transport in partial equilibrium that in the one a transfer of A from (o) to (i) is the result of a B-donator in (i) and a B-acceptor in (o), while in the other the transfer is the result of a B-donator in (o) and a B-acceptor in (i). It should be noted also that in Osterhout's model, B is not a carrier substance for A, although the donation and acceptance of B results in the transport of A.

Apparently both of these types of transport are involved in the theory of salt accumulation proposed by Lundegårdh (8). This theory is based on the assumption that the absorption of anions and absorption of cations are independent of each other to such an extent that different mechanisms are responsible for each.

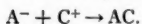
According to Lundegårdh, only the absorption of anions is coupled to the respiratory process. He postulates that

the Fe ion in the hemin group of a respiratory enzyme is well suited to effect an anion transport according to the scheme,

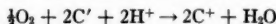


The trivalent Fe atom attracts one more anion than the bivalent Fe atom. If the enzyme system constitutes a structural unit, in which electrons move from one atom to another in the next molecule, the conditions are given for a transport of anions. The change of valency proceeds as an electron wave, if the enzyme system lies parallel to a redox gradient. If the molecules of the enzyme are arranged in such a way that they serve as a boundary between two media of a redox potentials, owing to the wave-like preceding oscillation of the Fe valency, they will transport anions from the medium with higher oxidation power (Fe^{+++} ; ox-side) to the medium with lower oxidation power (Fe^{++} ; red-side). The anions are transported in opposite direction to the electrons. The transference of an electron between two Fe atoms moves one anion from the oxidized to the reduced stage.

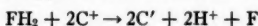
From the foregoing statement, it appears that Lundegårdh's mechanism for anion transport can be described in terms of the following model system: The chemical substances A^- , C^+ , H^+ , etc., are localized in two phases (i) and (o). A^- may signify any monovalent anion and C^+ corresponds to an oxidized hemin group of the cytochrome system (containing ferric iron). The phases (i) and (o) are separated by a connecting link M which contains the cytochrome system. The link M is impermeable to the solvent and to A^- , C^+ , H^+ , etc., but is permeable to a compound formed by the reaction



Also, the following reaction acts as a C^+ donator in (o)



where C' represents a reduced hemin group of the cytochrome system (containing ferrous iron), and (i) is connected to the C^+ accepting process:



where FH_2 is reduced flavoprotein.

In this model the compound AC will diffuse through the connecting link M from (o) to (i). Whether AC moves as an actual compound or the diffusion is accomplished by the passage of electrons and A^- in opposite directions is immaterial. By means of the diffusion of AC, A^- will be transported from (o) to (i) against a gradient in chemical potential. It will be noted that this model is essentially the same as that of Rosenberg for transport in partial equilibrium (Figure 1). Also, as pointed out by Robertson & Wilkins (14), the consumption of one molecule of O_2 should result in the transfer of four A^- ions according to this scheme.

In Lundegårdh's view, the accumulation of cations is accomplished through a completely different mechanism from that operating for anions. He concludes that since the protoplasm as a whole is negatively charged and contains appreciable quantities of substances with comparatively strong acid properties, cations in the culture medium can therefore exchange for H^+ ions in the plasma membrane and proceed inward through the protoplasm by exchange along paths or tracks of substances of acid dissociation. Thus by this process cations are transferred into the cell in exchange for H^+ ions which move out of the cell.

Apparently the accumulation of cations can be described in terms of the following model: The substances Z^+ , H^+ , HX , and ZX are localized in two phases (i) and (o). Also the following equilibrium obtains in each phase,

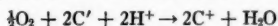


Z^+ signifies any monovalent cation and HX represents an organic substance of acid dissociation. The phases (i) and (o) are separated by a connecting link M which is impermeable to the solvent and to Z^+ and H^+ but is permeable to HX and ZX .

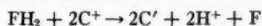
It will be noted that this model corresponds to Osterhout's model (Figure 3). The conditions of partial equilibrium are that μ_{HX} and μ_{ZX} are constant throughout the whole system and that

$$\mu_{\text{Z}^+}(\text{i}) - \mu_{\text{H}^+}(\text{i}) = \mu_{\text{Z}^+}(\text{o}) - \mu_{\text{H}^+}(\text{o}).$$

Now if phase (i) is connected to an H^+ -donator and phase (o) to an H^+ -acceptor, finite quantities of Z^+ can be transported from (o) to (i) against a gradient in chemical potential. These conditions are met if the reaction



takes place in phase (o) and the reaction



takes place in phase (i).

It will be noted that these are the same processes assumed for phases (o) and (i) to effect a transfer of anions from (o) to (i). Thus it is apparent that according to this interpretation the accumulation of anions and the accumulation of cations may depend on the same chemical reactions in the cell even though the transport mechanisms are different.

ION ABSORPTION BY ROOTS

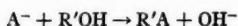
On the basis of the discussion thus far it is of interest to consider which of the proposed models will serve best as working hypotheses in the study of ion absorption by roots.

At the outset we are confronted with the fact that no method is available for ascertaining directly the composition of the vacuolar sap in this kind of tissue. For this reason, our conclusions must be based largely on the chemical examination of the culture medium and of the tissue as a whole. Nevertheless, the observations concerning mineral absorption by roots summarized earlier provide some ground for a choice of models.

It has been consistently observed, for low salt roots at least, that no appreciable quantities of ions are lost when root tissue is immersed in distilled water. This is the case even when the cells are not actively metabolizing (i.e., at lowered temperatures). This observation provides some grounds for assuming that the root cell is characterized by a connecting link (between the vacuole and culture medium) which is impermeable to free inorganic ions. Attendant upon this assumption is the supposition that the connecting link is permeable to certain organic compounds or complexes of the ions. Thus, the postulation of ion-binding or carrier substances appears necessary.

Since oxygen absorption is invariably associated with the accumulation of ions by roots, it is reasonable that the donation of the carrier substances on one side of the connecting link and their acceptance on the other must be accomplished through the agency of certain respiratory processes.

As stated earlier, ion absorption by roots takes place by way of exchange reactions between the culture medium and the tissues, and the absorption of cations is to some extent independent of the absorption of anions. In the light of these observations, the postulation of carrier substances of the types HR and R'OH seems appropriate. Thus, according to this idea, the initial step of ion absorption consists in the exchange reactions:



where Z^+ signifies a cation and A^- an anion.

Now if we assume that the carrier substances HR and R'OH are produced on the outer side of the connecting link, and further that the connecting link is permeable to ZR and R'A but not to Z^+ , A^- , R⁻ and R'⁺, we have adopted a model similar to that of Rosenberg for transport in partial equilibrium. The writing of R⁻ and R'⁺ does not imply that electrostatic bonds only are involved in the formation of ionic complexes. An additional necessary assumption is that the complexes ZR and R'A are destroyed on the inner side of the connecting link and the ions Z^+ and A^- are liberated to the vacuole in the forms of soluble salts or acids.

The above picture is essentially that adopted by Jacobson & Overstreet *et al.* (7). Also in regard to the carrier substances, it contains some of the

elements of the theory of Franck & Mayer (3). It will be noted that in this hypothesis the same model is assumed for the absorption of both anions and cations. This is in contrast to Lundegårdh's theory in which different models are assumed for the absorption of anions and cations.

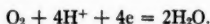
In the opinion of the authors, the experimental data for root tissue do not justify the assumption of different models for anion and cation absorption, although admittedly further experimentation may make such an assumption necessary.

As implied above, the symbols R and R' represent different organic groups. However, it is not known if HR (or R'OH) represents a single compound or a family of compounds, each of which possesses a high degree of selectivity for a particular cation (or anion). The problem of selectivity will have to be explained either in terms of different complexes for each ion or on the basis of different energies of formation utilizing the same binding compounds.

Of the various mechanisms of salt accumulation proposed in recent years, that of Lundegårdh (8) has probably received the most attention by plant physiologists. It is therefore appropriate to consider how adequately this theory will satisfy the requirements and known behavior of the accumulation process. The Lundegårdh scheme has already been described in this paper in some detail. Essentially it depends upon the transfer of electrons from an electron donor, i.e., reduced flavoprotein, to an electron acceptor, i.e., oxygen, via the cytochrome-cytochrome oxidase system. At the same time, the cytochrome system carries anions in the reverse direction. The hydrogen ion derived from the dehydrogenase activity moves out of the cell in exchange for a cation.

If the absorption is dependent upon the cytochrome system then it must be intimately related to that portion of the respiration mediated by the cytochrome system. This clearly appears to be the case. Spectrographic evidence obtained by Lundegårdh (8b) indicates that the cytochrome-cytochrome oxidase system functions in the roots of wheat and corn. Lundegårdh (8) and Milthorpe & Robertson (9) have shown that the increase in respiration occurring in the presence of a readily absorbed salt such as KCl (salt respiration) over that occurring in distilled water (ground respiration) is inhibited by cyanide and azide. Inhibition by these substances frequently, but by no means invariably, suggests that the cytochrome system is involved. Recently more clear-cut evidence became available for the participation of the cytochrome system in salt respiration and accumulation. Weeks & Robertson (20) showed with carrot disc that both salt respiration and accumulation were inhibited by CO in the dark and that this inhibition was reversed in the light. Similar evidence was obtained by Sutter (18a), and Lundegårdh (8a, 8b) has developed techniques and made direct measurements of the absorption spectra of reduced and oxidized cytochrome in roots. These results offer the most direct evidence of a close relationship between accumulation and cytochrome oxidase. Another type of evidence is

that dealing with a quantitative relation between salt accumulation and the functioning of the cytochrome system. For every molecule of oxygen reduced, four electrons are consumed:



If, therefore, one anion is accumulated for each electron transferred by the cytochrome system, then for each molecule of oxygen utilized in salt respiration, four anions should be accumulated, assuming 100 per cent efficiency. Robertson & Wilkins (14) working with carrot tissue, have obtained values close to four, although considerably lower values have been obtained with other tissues such as wheat roots.

Although the light reversible inhibition by CO and the correspondence between the number of anions and the number of electrons transferred by the cytochrome system is strong presumptive evidence for the Lundegårdh scheme, they are not entirely conclusive. A complicating factor is the inhibition of salt accumulation by 2,4-dinitrophenol (DNP) which does not inhibit the cytochrome system but rather interferes with the transfer of energy-rich phosphate in phosphate coupled oxidations. Roberts, Roberts & Cowie (12) showed this inhibitory effect on salt absorption in *E. coli*, and more recently Robertson, Wilkins & Weeks (15) have demonstrated the inhibitory effect of DNP on salt accumulation in carrot tissue although the cytochrome-mediated respiration was not inhibited. It is rather difficult to fit the DNP effect into the Lundegårdh hypothesis, as presently postulated, without some further assumptions or extensive modifications.

Other objections which have been raised concern the widely differing rates of absorption of different ions of the same charge, the unequal rates of absorption of a cation and its associated anion, and the mutual reciprocal effect of ion pairs. These observations can be explained in part according to the Lundegårdh hypothesis but, again, not without making other assumptions.

Perhaps the greatest service of the Lundegårdh scheme has been in showing the very close relationship existing between salt accumulation and the cytochrome system. In view of the quantitative and qualitative linking of accumulation with the cytochrome-mediated respiration, it seems clear that any theory of absorption will necessarily involve the cytochrome system either directly or indirectly. However, the identification of the cytochrome-cytochrome oxidase system with the actual ion carrier seems premature on the basis of the presently available evidence.

A similar criticism may be directed against the postulations of Brooks (1) and of Steward & Street (18). Brooks suggested that amino acids might serve as carrier substances for anions and cations, the H^+ of the $-\text{COOH}$ being exchangeable for a cation and the OH^- of the hydrated amino group, i.e. $-\text{NH}_2\text{OH}$, being exchangeable for anions. According to this idea, a transference of ions from the external phase to the internal phase could occur by means of a series of exchanges involving H^+ and OH^- . Steward & Street (18)

observing that protein synthesis frequently, if not invariably, accompanies ion accumulation, speculated that certain energy-rich phosphorylated nitrogen compounds in the protoplasm might function as carriers for the ions.

Recently Jacobson & Overstreet (6) have proposed a general formulation of the ion absorption process, without, however, attempting to identify the ion carrier. Nevertheless certain observations can be made concerning the nature of the ion carrier. These may be summarized as follows: (a) The ion carriers are intermediate metabolic products or closely related substances; (b) the carriers are not stable *in vitro*; (c) they undergo chemical alteration in the course of their carrier function; and (d) they probably function as chelated complexes.

The well known close dependence of ion absorption on metabolic activity strongly suggests that the transport of ions is coupled more or less directly to the metabolic process, i.e., the carrier substance is produced during these processes.

Efforts to isolate from barley roots a substance capable of forming complexes with such ions as potassium have not been successful even when carried out at low temperatures. It is probable, therefore, that the ion carrier for these ions is extremely labile.

In order to account for secretion of ions into the vacuole, it is necessary to postulate a decomposition of the complex in such a way that the substance can no longer serve as a carrier. Also, the chemical alteration is a necessary requirement of the model previously postulated.

The only known bonds capable of holding potassium and similar ions with the required binding strength are chelate bonds. Hutner (5) and others have pointed out that ions may be bound in living cells in this fashion. It should be emphasized that this formulation, as are all others, is based on a model. It is questionable that a simple model can accurately represent the process of ion accumulation as it occurs in a living cell. This, however, does not detract from the value of models as a basis for further experimentation.

In spite of the fact that a direct study of the carrier substance may not be possible, indirect studies are of considerable use in evaluating and characterizing the carrier substance. Such an indirect study is that which deals with competition between ions.

COMPETITION BETWEEN IONS

It is of considerable importance to know if the cation complex forming substance HR represents a single compound which serves for all cations or whether it represents a family of compounds, each one of which is more or less specific for a particular cation. These same considerations also apply to the anion complex forming substance R'OH. Attempts have been made to elucidate this problem by means of studies of pairs of cations, the idea being that the presence of competition between two ions would indicate a single binding substance and the absence of competition would indicate separate binding substances. Studies of this nature are contained in the work of

Jacobson *et al.* (7), Overstreet, Jacobson & Handley (11) and Epstein & Hagen (2).

Jacobson *et al.* (7) studied the interaction between H^+ and K^+ . Their findings were consistent with the view that the primary step in absorption could be expressed as:



This reaction could be driven to the right or left, depending upon the ratio of K^+ to H^+ in the external solution. In these experiments no absorption of K^+ was observed if the ratio of K^+ to H^+ was less than 17 to 1.

Marked competitions in absorption were noted between Na^+ and K^+ . This has been interpreted to indicate a single binding compound for these two ions.

Overstreet, Jacobson & Handley (11) studied the competition between Ca^{++} and K^+ . The results were unusual in that the presence of K^+ markedly reduced the absorption rate of Ca^{++} , whereas the presence of Ca^{++} in certain concentration ranges reduced the absorption rate of K^+ and in other concentration ranges markedly increased the absorption of K^+ . Overstreet *et al.* conclude that probably a single binding substance serves for both K^+ and Ca^{++} , but in addition a special role must be assigned to Ca^{++} in the absorption process. They conclude that Ca^{++} is effective in the removal of the complex KR from the site of the absorption reaction.

Epstein & Hagen (2) have compared the absorption process with an enzymic reaction. They consider the reaction of an ion with a binding substance as analogous to the combination of a substrate with an enzyme. In their treatment, interfering ions assume the role of inhibitors or alternate substrates. They found that K^+ and Cs^+ interfered competitively with Rb^+ absorption and concluded that these three ions were fixed by the same binding sites or reactive centers. Na^+ , on the other hand, except at high Rb^+ or Na^+ concentrations, does not interfere competitively with Rb^+ absorption, and presumably not with K^+ absorption. This is in contrast with the conclusions of Jacobson *et al.* Li^+ did not compete with K^+ , Rb^+ , or Cs^+ , and at low concentrations of Rb^+ and Li^+ the absorption of Rb^+ was increased.

ENERGY RELATIONS

A significant aspect of the models for transport in partial equilibrium is that no work is done by the model in such transport. This is obvious from the fact that the models always operate under conditions of equilibrium. An equivalent interpretation is that the work effects associated with the transport of a solute (A in the models) from a lower to a higher chemical potential are offset by the work effects associated with the transport of the carrier substance (B in the models) from a higher to a lower chemical potential. However, as pointed out earlier, a finite transport of A requires the co-operation of the surroundings in that finite quantities of B must be donated in one phase and removed in the other phase. This can only be accomplished

by virtue of certain processes in the environment which take place spontaneously, i.e., occur with a decrease in free energy. According to this picture, although the model itself does not perform work in active transport, a decrease of free energy, nevertheless, must occur in the surroundings.

For a given period of time, the transport of ions in the model is related to the amount of carrier substance produced. However, the amount of transport will bear no relation to the free energy changes of the reactions donating and accepting the ion carrier. For this reason, the expression of energy relationships appears meaningless. A more logical expression of the efficiency would be in terms of the amount of solute transported by the model per unit of carrier substance donated by the environment. In general, the rate of a chemical reaction bears no relation to the change in free energy of the reaction. Thus, the significant feature is not the changes in free energy, but rather the rates of the reactions producing and removing the ion carrier.

It should be emphasized that the foregoing consideration applies only to the models previously described. In a living cell, other considerations would have to be taken into account, such as the expenditure of chemical energy necessary to synthesize a connecting link between the inner and outer phases possessing the required properties.

CONCLUSIONS

A survey of a number of suggested mechanisms for ion accumulation by plant cells indicates that the process can best be elucidated in terms of models for Rosenberg's "transport in partial equilibrium" (16). These models are characterized by a connecting link between two solutions which is essentially impermeable to free ions and the solvent but relatively permeable to the ion-ion carrier complex. The transport of ions depends upon the donation of the ion carrier in one phase and its removal in the other phase. In order to obtain finite transport of ions from one phase to another it is required that the transport be related to certain spontaneous chemical reactions in the environment. A significant attribute of such a model is that no net work is performed by the model during active transport, although for such transport certain reactions must occur in the environment which proceed with a decrease in free energy. Moreover, this decrease in free energy need bear no relation to the quantity of ion transported.

The identification of the mechanism of salt absorption in roots with a model for transport in partial equilibrium raises questions regarding the ion carrier: namely, its origin, its identity, and its fate. The strict dependence of absorption on aerobic respiration strongly suggests that the ion carrier is produced in that phase of the respiratory process. The evidence favors the view that the formation of the ion carrier is closely linked with the operation of the cytochrome system. However, some doubt attends the identification of the actual ion carrier with an oxidized member of the cytochrome system.

It has not been determined whether or not the carrier substance HR represents a single compound with differing affinities for different cations or

a series of more or less specific binding compounds. The evidence seems to favor a single compound or at most relatively few compounds. At the present time, there is little or no evidence bearing on similar questions concerning the anion carrier R'OH.

LITERATURE CITED

1. Brooks, S. C., *Trans. Faraday Soc.*, **33**, 1002-6 (1937)
2. Epstein, E., and Hagen, C. E., *Plant Physiol.* (In press)
3. Franck, J., and Mayer, J. E., *Arch. Biochem.*, **14**, 297-313 (1947)
4. Hewitt, E. J., *Ann. Rev. Plant Physiol.*, **2**, 25-52 (1951)
5. Hutner, S. H., *Trans. N. Y. Acad. Sci.*, **10**, 136-41 (1948)
6. Jacobson, L., and Overstreet, R., *Am. J. Botany*, **34**, 415-20 (1947)
7. Jacobson, L., Overstreet, R., King, H. M., and Handley, R., *Plant Physiol.*, **25**, 639-47 (1950)
8. Lundegårdh, H., *Arkiv Botan.*, [A]**32**, 1-139 (1945)
- 8a. Lundegårdh, H., *Arkiv Kemi*, **3**, 69-79 (1951)
- 8b. Lundegårdh, H., *Arkiv Kemi*, **3**, 469-94 (1951)
9. Milthorpe, J., and Robertson, R. N., *Australian J. Exptl. Biol. Med. Sci.*, **26**, 189-97 (1948)
10. Osterhout, W. J. V., *Cold Spring Harbor Symposia Quant. Biol.*, **8**, 51-62 (1940)
11. Overstreet, R., Jacobson, L., and Handley, R., *Plant Physiol.* (In press)
12. Roberts, R. B., Roberts, I. Z., and Cowie, D. B., *J. Cellular Comp. Physiol.*, **34**, 259-91 (1949)
13. Robertson, R. N., *Ann. Rev. Plant Physiol.*, **2**, 1-24 (1951)
14. Robertson, R. N., and Wilkins, M. J., *Australian J. Sci. Research*, [B]**1**, 17-37 (1948)
15. Robertson, R. N., Wilkins, M. J., and Weeks, D. C., *Australian J. Sci. Research*, [B]**4**, 248-64 (1951)
16. Rosenberg, T., *Acta Chem. Scand.*, **2**, 14-33 (1948)
17. Spiegelman, S., and Reiner, J. M., *Growth*, **6**, 367-89 (1942)
18. Steward, F. C., and Street, H. E., *Ann. Rev. Biochem.*, **16**, 471-502 (1947)
- 18a. Sutter, E., *Experientia*, **6**, 264 (1950)
19. Teorell, T., *Proc. Natl. Acad. Sci. U.S.*, **21**, 152-61 (1935)
20. Weeks, D. C., and Robertson, R. N., *Australian J. Sci. Research*, [B]**3**, 487-500 (1950)

PHYSIOLOGICAL BASES FOR ASSESSING THE NUTRITIONAL REQUIREMENTS OF PLANTS¹

BY ALBERT ULRICH

University of California, Berkeley, California

Plant analysis, when used as a means of assessing the nutritional requirements of plants, may be envisaged in its simplest terms as a study of the relationship of the nutrient content of the plant to its growth. This relationship may be ascertained in several ways—by empirical means, through plant physiological considerations, or by a combination of the two procedures. From a strictly empirical point of view, the nutrient content of the plant could be studied in relation to growth without regard to basic concepts in plant physiology. From a purely plant physiological approach to assessing the nutritional requirements of plants, progress in plant analysis would be made only when the function of each nutrient involved was firmly established. Since only a few of the functions of the nutrients required for plant growth or their reactions are known at present, a reasonable approach to the solution of the problem of plant analysis would appear to be to utilize the physiological information now available and to proceed therefrom on a trial and error basis. That this combined approach has been successful may be readily shown by the progress made over the years in applying plant analysis for appraising the nutrient requirements of crops in experimental plots and in commercial fields (1 to 13, 19, 22, 23, 30, 35, 36, 72, 73, 91, 92). It is the purpose of this review to note this progress, to consolidate gains that have been made, and to refer to the literature only so as to provide continuity of the subject matter under discussion. Excellent reviews of plant analysis by Salter & Ames (14), Goodall & Gregory (18), Thomas (15, 16), Ulrich (19), Hewitt (20), Nicholas (21), Lilleland (17), Boynton & Compton (34), and Lundegårdh (12) have been published elsewhere, and these may be consulted for further details as they apply to this specialized field of plant nutrition.

HISTORICAL

The earliest work on plant analysis, reported by de Saussure in 1804 (24), was motivated by a curiosity concerning the ash content of plants. He showed through his studies that the ash content of plants varied with the plant, its age, the plant part, and the soil on which the plant grew. No attempts were made by him to relate the nutrient content of plants to growth or to their nutritional requirements. Liebig (25) in 1852 suggested that the fertility of a soil could be maintained through the simple expedient of returning to the soil as fertilizers the nutrients contained in the crops removed from the field. Later knowledge concerning the fixation of phosphate and potas-

¹ The survey of the literature pertaining to this review was concluded in December, 1951.

sium by soils in forms that are unavailable to the plant and information about losses of nitrogen to drainage waters and to the air as a gas, proved this simple hypothesis to be untenable. Hall (26) at Rothamsted in 1905, envisaged plant analysis as a method for estimating the nutrient content of soils, which he felt could be accomplished only after establishing the *normal nutrient content* of the plant (crops or weeds) through numerous chemical analyses. Any deviations from the normal could then be used as a guide to the fertilization of the crop to be grown on the field. However, he concluded from his observations on root crops and cereals that "pending the determination of phosphoric and potash 'constants' for some test plant occurring naturally on unmanured land the interpretation of soil conditions from analyses of plant ashes is not a practicable method by which chemical analysis of the soil can be displaced." Furthermore, he found that even though the proportion of phosphoric acid and of potash in the ash of any given plant varied with the amount of these substances in the soil, the variations depending on season or differences in supply of non-essential ash constituents may be as great as those from the fertilizations. These observations demonstrated to him and to others (14, 27) that the successful use of plant analysis for estimating the nutrient-supplying power of soils was not within reach.

It was almost twenty years after Hall's report that plant analysis was again considered seriously by investigators in the field of plant nutrition. The work at this time fell into two distinct categories. One group of workers represented by Gilbert & Hardin (28), Gilbert, McLean & Adams (29), and Emmert (31) analyzed fresh plant material, while another group analyzed dried plant material, [Pfeiffer, Simmermacher & Rippel (32), Lagatu & Maume (33), and Mitchell (35)]. Since then much progress has been made in the techniques and in the interpretation of the results in both directions.

DEVELOPMENT AND SELECTION OF TECHNIQUES

Analysis of fresh versus dried plant material.—In using fresh plant material, tests can be made directly in the field. Hoffer (37) was the chief proponent of this procedure. He developed the indirect test for potassium by testing for iron in the conducting tissue of corn stalks, and the direct test for nitrate with diphenylamine reagent. Many improvements were made in these methods, particularly for potassium, and these culminated in the "Purdue test kit," as developed by Thornton *et al.* (39), which is now used extensively in the field on various crops for nitrate, phosphate, and potassium. One of the important findings through tissue testing in the field is the great variability of individual plants growing under apparently uniform conditions of soil and climate (5). This variability is particularly noted in plants in a field that is verging on a deficiency of one or more nutrients. For example, one plant will be found to have depleted its nitrate supply while another adjacent to it will still have an ample supply as shown by a high nitrate test, thus indicating that the latter plant was more favorably situated with respect to nitrogen availability in the soil, or that its early growth had

been more advantageous and thereby had absorbed a larger proportion of the available nitrogen than its neighbor. In view of the great variability of plants and soils, it would be remarkable indeed if all plants became deficient in nutrients at the same time.

Testing of plants in the field is, of course, quite inaccurate, and in actual practice serves only as a rough guide for determining the nutrient status of a crop. For greater accuracy and for an over-all saving of time, the analysis of fresh plant material, whenever possible, is done in the laboratory (22, 23, 30, 31, 40, 41, 78). In using either field or laboratory methods of analysis, only a short period of time is required from the time of taking the samples and the time of securing the analytical results. This saving in elapsed time is often of considerable importance in the commercial testing of field crops, but unfortunately, fresh material has the disadvantage of being labile and therefore must be analyzed promptly, or at best stored at low temperatures for short periods of time (19). Then, too, the moisture losses that often take place in plant samples during sampling or en route to the laboratory are of no consequence if plant material is to be dried, but for fresh material even slight losses in moisture will affect the results to be reported. For this reason fresh material for laboratory analysis must be stabilized immediately in the field or placed in air tight containers which are then kept cool until the samples are analyzed. The time of day for collecting the plant samples should be standardized with considerable care, particularly for samples to be analyzed fresh, since this timing will minimize the errors resulting from the diurnal fluctuations in the moisture and nutrient content of the plants verging upon deficiency.

Plant material for drying must be dried promptly in a well ventilated oven, preferably at 70°C. Once the material is dry, it may be stored indefinitely, either unground or ground, awaiting convenient scheduling for analysis or the completion of analysis of other constituents that may add much information about the nutrient status of the plants under study. Dried material has the further advantage that moisture losses, during sampling or in transit to the laboratory, do not affect the analytical values obtained. From these considerations it is quite clear that the use of dried plant material is preferable to fresh material in all instances except when the results are needed immediately after taking the plant samples, and even here the gain in time may be only from eight to twenty-four hours in favor of the fresh plant material.

Selection of plant part.—The part of the plant to be used for chemical analysis depends upon the objectives in view. From a strictly diagnostic point of view, only tissues with deficiency or toxicity symptoms need to be collected in amounts sufficient for chemical analysis. If the nutrient values found within the plant are comparable to those of plants with known deficiencies, then a decision can be made as to the most likely cause for these symptoms (8, 18, 19). Final proof of the correctness of the diagnosis, however, may be deferred until the symptoms fail to appear on the treated plants in comparison with those plants left untreated.

When the objective of plant analysis is to serve as a guide to the fertilization of field-grown crops, the solution of the problem is much more complicated. In attempting to attain this goal, many investigators have analyzed many tissues, ranging from the entire tops of plants to clearly defined parts of a given plant. When the entire tops of plants are taken as the sample, it is quite clear that many tissues, differing in age and function, are included. Naturally, upon the chemical analysis of such material an average condition for that plant is obtained which will depend upon the nutrients available from the soil, rate of growth of the plant, and upon the proportion of each tissue to the others present. For example, the differences in proportion of embryonic to older tissues in plants might very well account for the anomalous results observed by Piper (42, Table 2) for oats, and by Steenbjerg (43, Figure 4) for barley, in the copper concentration of the tops of the plants deficient in copper at different stages of growth. They found that the most deficient plants had a higher copper concentration than larger plants that were also copper deficient. Thereafter, as more copper was added to the soil, the copper deficiency became less pronounced, and the copper concentration of the plants, which now included leaves and stalks, again increased. This same phenomenon has been observed by Stout & Pearson (44) with corn plants treated with radio-zinc. In their experiments the plants, which were extremely deficient in zinc, had a higher zinc concentration in the tops than larger plants also deficient in zinc. The radio-autographs of the corn plants revealed that the zinc had accumulated in the growing points and in the nodes of the plants (Figure 1). Furthermore, it was noted that with larger plants, produced by adding a little more zinc to the culture solutions, the internodes which did not accumulate zinc increased greatly in length and accounted for most of the increased growth. From these facts it may be concluded that as the tops of the plants increased in size, the proportion of internodal to nodal and embryonic tissues increased, and accordingly, the zinc concentration of the entire tops decreased. However, when more than enough zinc was available to satisfy plant requirements the zinc concentration of the tops was also high. It is quite likely that a separate analysis of either nodal or internodal tissues would give a better indication of the zinc status of corn plants than an analysis of the entire tops. This point should be determined in future studies for corn plants with zinc and for cereals with copper.

While the use of radioactive nutrient elements has demonstrated dramatically their distribution in plants, the uneven distribution of plant nutrients was observed some time ago by plant physiologists, as, for example, potassium in tomato plants by Janssen & Bartholomew (58), Arnon & Hoagland (47), and Ulrich (19), and in tobacco plants by Drake & Scarseth (45). When these plants were low in potassium, the highest potassium concentrations were found in the growing points, and the lowest in the lower portions of the plant. When the plants were high in potassium the reverse situation prevailed, and the highest concentrations were now in the basal



FIG. 1. Radioautographs of zinc (Zn^{65}) in a corn stalk near the tassel (left), and immature ear of corn (right), showing the accumulation of radio zinc in the nodes and in the seeds of the corn plant. (Unpublished results of Stout and Pearson, 44.)

leaves of the plant, and the lowest concentrations, although still quite high, were found in the tissues near the apex of the plant. The selection of recently "matured" leaves for analysis, located about a third of the way down from the apex of the plant, would circumvent these extremes in potassium concentration, and at the same time would emphasize the tissues contributing actively to the growth of the plant.

For grape vines, Lagatu & Maume (33) recommended the selection of leaves of the same physiological age. They suggested plucking basal leaves of canes, which it is true, would have the same physiological age at each

sampling period, but as the season progressed would become of less importance to the entire plant. This difficulty may be avoided, as mentioned, by selecting a recently "matured" leaf just below the tip of the cane for the sample (19, 48). For pineapple plants, Nightingale (2) employed leaves at a precise stage of growth, in which the leaves "... are almost fully expanded, except for the lateral points at the base, which in a fully mature leaf approaching senescence form a much more acute angle, and in an immature leaf form approximately a right angle. It is thus simple always to select exactly comparable leaves for analysis, regardless of environmental influences or whether the plant has been growing for three months or a year. . . ." Instead of taking the entire pineapple leaf for analysis, the white semi-meristematic basal tissue of the leaves was analyzed (2); for grape vines the petioles of recently "matured" leaves have been found to reflect the potassium status of the vines more clearly than an analysis of leaf blades (48). Preference of petioles over blades in grapes held also for nitrate (49) and phosphate (19); for raspberries (89) this was also true for phosphorus, potassium, and calcium. For potatoes, petioles (rachises) from definite positions on the plant were selected for analysis by Lorenz (77), Hill & Cannon (76) and by Terman *et al.* (75). For other crops and for other nutrients, the greater sensitivity of petioles over blades for nutrient changes within the plant may not always hold, as was indicated recently by Chapman & Brown (8) for potassium in navel orange leaves, and by Ramig & Vandecaveye (89) for nitrogen and magnesium in raspberry leaves, or be consistent enough to furnish a reliable index of potassium requirements, as concluded by Frear *et al.* (87) for peaches. Materials suitable for analysis have been suggested for sugar cane by Clements & Kubota (4), for apples by Boynton & Compton (34), for prunes, apricots, almonds, apples, pears, and peaches by Lilleland (17), for citrus by Chapman (54), for sugar beets by Ulrich (19, 50) and Brown (51), for cauliflower and potatoes by Nicholas (22), for peas by Tremblay & Bauer (83), and for many crops by Goodall & Gregory (18) in their review of plant analysis.

Early in the history of plant analysis it was found that the ash content of fruit varied considerably less than the vegetative portions of the same plant. In 1905, Hall (26) observed that the composition of the ash of grain of cereals or tubers from potato plants varied considerably less than their corresponding vegetative parts. Similar observations have been recorded for oats by Burström [quoted by Lundegårdh (12)], for tomatoes by Arnon & Hoagland (47), for corn, soy beans, and wheat by Lucas *et al.* (74), and for peaches by Frear *et al.* (87). For tomatoes, relatively small changes in the mineral composition of the fruit were observed by Lyon *et al.* (69), and in vitamin C content by Hamner *et al.* (68), even though the solutions used for culturing the plants differed widely in mineral composition. Accordingly, from the viewpoint of determining the nutrient status of crops, little would be gained from the analysis of fruiting bodies. Neither does the analysis of entire plants appear promising. Much greater success appears to lie in the

direction of selecting specific parts of the plants such as portions of the stem, petioles, or blades from specific leaves taken from definite parts of the growing plant. The part of the plant to be used must be determined not only for each crop, but should also be established for each nutrient to be analyzed.

Plant sampling.—The actual sampling of plants with deficiency or toxicity symptoms is again rather simple and consists merely of taking for analysis adequate amounts of material that have recently developed characteristic symptoms. Sampling of plants in order to estimate the nutrient status of the crop while growing in the field is more complicated, and depends upon the crop and upon the size of the area under observation. The first requisite for field sampling is an adequate amount of material for analysis, and the second that the material collected actually represents the plants or area sampled. For individual trees, Lilleland (17) has recommended the collection of 100 leaves around the tree at shoulder height. For groups of ten trees, ten leaves per tree were used. Similar procedures have been recommended by Drosdoff (71) for tung, by Chapman (54), Chapman & Brown (8), Reuther *et al.* (79) for citrus, and Boynton *et al.* for apples (88). For grapes in experimental plots two rows wide and twenty vines long, four petioles spaced at equidistances around each vine for all forty vines, reflected accurately the nutrient status of the vines in each plot. For very large plots of 100 or more grape vines, one petiole from every vine, or every other vine has been found adequate (19). Procedures for sampling sugar beets have been outlined by Brown (51) and Ulrich (10, 19), for pineapple by Nightingale (2), for sugar cane by Clements (11), for potatoes by Lorenz (77), Terman *et al.* (75), and Hill & Cannon (76), and for many crops by Carolus (78).

Time of day.—The time of day for collecting plant samples has not been considered of great importance by most investigators in the field of plant analysis. Nightingale (2) stated that samples from pineapple plants were collected from 8:00 a.m. to 4:00 p.m., and Mitchell & Chandler (36) recommended 9:00 a.m. to 3:00 p.m. for forest trees, while the reviewer has taken samples at all hours of the day. In one experiment in a field of sugar beets, relatively high in nutrients, there were no large differences in the NO_3 , PO_4 and K content of petioles collected at two-hour intervals from sunrise to sunset and again at sunrise the following day. If the beets sampled had been very low or deficient in a nutrient, then significant differences in nutrient concentration might have been observed, particularly for nitrate. However, it is not likely that the fluctuations that would have been observed in a deficient plant would have been large enough to classify the plants as being amply supplied at one time, and below the critical concentration the next. This conclusion is supported by observations from hundreds of fields sampled; those classified as deficient in NO_3 were actually found to be deficient, and those classified as high were actually found to be high, regardless of the time of day the plants were sampled.

Form of nutrient.—Nutrient elements essential for the growth of plants may be present within the tissues of the plant in one dominant form, as for

example, ionic potassium, or in many different forms or compounds, as observed for nitrogen and phosphorus. The early analyses of plants for evaluating the nutrient status of crops or soils were concerned primarily with determining the total concentration of the element present within the plant. The results of these analyses were expressed in per cent of the dry matter, or more frequently as the percentage composition of the ash found within the plant (52). It was only upon the analysis of fresh plant material that it became easier to determine the nutrients soluble in plant solutions (29, 59), in dilute acids (38, 53, 78), or in buffered salt solutions (22, 75) than in the total plant material. By analyzing the plant for soluble nutrients, the concentrations of the raw materials within the plant would be given prior to their elaboration into protoplasm and structural tissues, and thereby an estimate of the nutrient status of the plant at the time of sampling could be made readily (5). Under these conditions emphasis is placed upon the nutrient status of the plant rather than upon the nutrient content of the soil, an important departure from the viewpoint of Hall (26), Münter (27), Salter & Ames (14), Mitchell (35), and Mitchell & Chandler (36).

The advantage of determining the unelaborated compounds in plants was extended by Ulrich (49) to dried plant material for nitrogen in grape leaves. Here a comparison of nitrate nitrogen, soluble nitrogen (non-colloidal), insoluble nitrogen (primarily proteins), and total nitrogen on blades and petioles showed that the nitrogen status of grape vines could be estimated much better through a nitrate nitrogen determination on petioles than a determination of nitrate nitrogen on blades or by a determination of other nitrogen fractions on either petioles or blades. The analyses of sugar beet petioles were also in agreement with these findings (50). Similarly, for phosphorus, phosphate phosphorus soluble in 2% acetic acid was found for Ladino clover (55), sugar beets (56), and for grapes (82) to be preferable to the determination of total phosphorus concentration in the dried plant material. Since all or nearly all of the potassium in dried plant material is readily extractable by water (58), the analysis of the plant material for total potassium has been generally adopted. For other nutrients essential to the growth of plants, the fraction of the element best for analysis must still be determined.

Seasonal trends.—The influence of time, or more correctly, the effect of age upon the nutrient content of leaves, is brought sharply into focus every time a series of samples are collected from comparable plants during the growing season (Figure 2). Generally speaking, the concentrations of most nutrients, expressed on a dry basis, tend to follow a similar pattern, that is, they are higher during the early growth periods of the plant and gradually decline thereafter as the plants develop to maturity. Part of this gradual decline is a reflection of the increase in the proportion of structural material to protoplasm within the plant (greater percentage of dry matter), and another part is due to the gradual decrease in supplying power of the soil for nutrients. Many exceptions to this general rule, however, may be observed, par-

ticularly for such nutrients as calcium (46, 60, 81, 85, 86) and magnesium (4, 60, 85), which generally rise with the age of the leaves, and for plants on

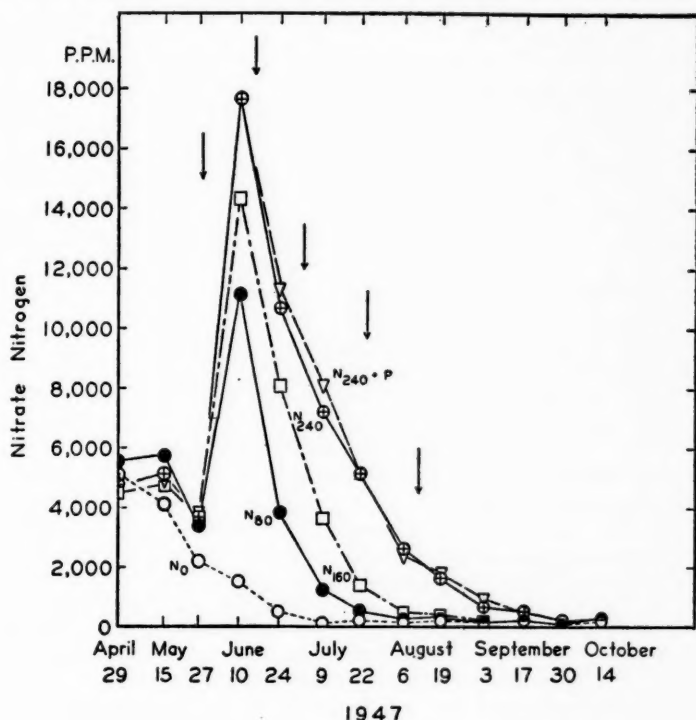


FIG. 2. Nitrate-nitrogen (dry basis) of recently "matured" petioles of sugar beet plants for field H as related to the amount of nitrogen applied, date of irrigation and date of leaf sampling. N=pounds of nitrogen per acre applied as ammonium nitrate in the amounts indicated by the numbers in the subscripts thereto and P=200 pounds per acre of P_2O_5 added to the soil as treble super phosphate. The arrows indicate the dates of irrigation subsequent to the fertilization of May 9. Each point is an average of eight replications.

The samples collected on April 29, 1947, prior to fertilization, did not differ significantly in nitrate concentration. After fertilization, but before irrigation, the samples collected on May 15 and May 27 still did not differ significantly from each other. However, after water was applied on May 28, the petioles collected on June 10 had increased greatly in nitrate wherever ammonium nitrate had been applied. The average beet root yields were 16.3, 22.1, 23.8, 23.8 and 23.9 tons per acre for the N_0 , N_{80} , N_{160} , N_{240} , and $N_{240}+P$ plots. A difference of 1.3 tons per acre is necessary for significance at the 5% point (10).

soils not sufficiently fertile to supply the plants with an adequate amount of all nutrients necessary for growth. Thus, for plants on soils deficient in nitrogen, potassium may accumulate in the plants even though the plants will become deficient in potassium as soon as the soils are fertilized with an adequate amount of nitrogen. Not only do the nutrient concentrations of plants change during the growing period for any one season (2, 3, 9, 15, 19, 22, 33, 34, 46, 48, 61, 67, 72, 73, 80, 81) but there may be profound differences in plants from year to year growing on the same site (26, 34, 48, 60, 67). Part of this yearly variation is undoubtedly associated with the normal variation in climate and part to phenomena which occur rather infrequently, such as killing frosts, wind storms, droughts, floods, severe diseases, heavy insect infestations, etc. In this connection Cain & Boynton (86) found the leaves of apples to have a higher potassium concentration during a no-crop year caused by frost damage to the blossoms than in the preceding year of heavy crop. Variations in the potassium content of alternate bearing trees may be quite large as shown by Lilleland & Brown (60) for prune trees having part of their crop removed by thinning early in the growing season, or by comparing trees differing greatly in number of fruit. Similarly, Arnon & Hoagland (47) observed, by removing the flowers of tomato plants low in potassium, that the potassium concentration of the leaves was raised in comparison to comparable plants with fruit.

Frequency of sampling.—The number of samples taken from a crop during the growing season will depend upon the number of samples required to show clearly the nutrient changes taking place within the plants as they develop from a seedling to the mature plant. Once these trends are known for a particular crop in a given area, then frequently the nutrient status of that crop can be estimated adequately from only one set of samples taken at the proper time of the year, while for other crops, two or more samplings will be required during the growing season. For short-term crops such as lettuce and spinach, weekly samplings may be necessary at first, and for sugar beets (19), a sample taken every two to four weeks is desirable initially, but later may be reduced to three, and possibly two, samplings per year. For vine (33, 61) and tree crops, (86) monthly samplings are sufficient for initial studies, and later, for routine checking and for surveys, sampling may be reduced to one per year; for example, mid-summer for grapes (61), middle of June to the middle of August for deciduous fruits in California (17), July 15 to September 1 for fruit trees in New York (34), July through October for orange trees in California (8, 54), August for tung trees (71), and at the time of flowering for oats (12).

Generally, the time selected for conducting a plant nutrient survey is a compromise between early sampling, when few plants will be found deficient and late sampling, when more plants will be found deficient (Figure 2) but will be less likely to respond to added nutrients either during the current season or in the following year unless the deficiency occurs regularly early in the growing season (19).

INTERPRETATION OF RESULTS

In developing plant analysis as a means of assessing the nutritional requirements of plants, the analytical methods used have been improved greatly during recent years, although progress in the interpretation of the results has not been so well defined or established. Part of the difficulty has been in the failure to define clearly and consistently the objectives of plant analysis and partly in not appreciating fully the many factors involved in attaining each objective. Historically, Liebig (25) used plant analysis as a guide to fertilizing the soil with nutrients in accordance with the amounts removed by the crop, while Hall (26) hoped to utilize plant analysis as an indirect means of analyzing the soil. Neither objective has been realized and the reason for failure in each instance has been stated earlier in this review. More recently, however, the major objectives of plant analysis have been to predict the nutrient requirements of crops on the basis of a single sample taken in mid-season (12, 19, 63, 83), and to evaluate the nutrient status of the crop for that particular time of plant sampling (8, 9, 19). In both instances the concept of the critical nutrient concentration is generally involved.

CRITICAL NUTRIENT CONCENTRATION

Development of the critical nutrient concept.—At the present time the most fruitful approach to the interpretation of results of plant analysis is through an association of the nutrient concentration of the plant with the growth of the plant made up to the time of sampling. Theoretically, this relationship may be linear, curvilinear, or possibly even intermittently linear or curvilinear, depending upon whether the nutrient element being studied is required continuously or for specific stages in the development of the plant. If the function or functions of each element were completely, or even partly, known, then the exact concentration just sufficient for maximum growth could be estimated correctly. In the absence of this information, the nutrient concentrations required for maximum growth must be estimated empirically.

The empirical approach to evaluating the nutrient status of crops has evolved gradually. Gilbert (59), in relating the nutrient concentration of plants to growth envisaged many of the principles which appear pertinent to the empirical solution of this problem. While he and his colleagues (28, 29, 59) analyzed the "juice" of the tops of plants and often thought in terms of the nutrient content of the normal plant, nevertheless, they considered that the soluble nutrients found in the plant served as raw materials for the elaboration of plant foods; that the concentration of nutrients found within the plant at any one time was the balance between the supply from the soil and the amount utilized by the plants; and that certain nutrient concentrations were suggested as being critical for the growth of the plants studied. Of particular encouragement to them was the observation that the addition of a fertilizer to the soil resulted in an increased content of that nutrient in the plant, and that this was associated with an increase in yield

when the nutrient levels were sufficiently low in the plants (28, 29). The latter observation relating plant growth to internal nutrient concentrations was made independently somewhat earlier by Pfeiffer, Simmermacher & Rippel (32) and by Lagatu & Maume (33) at about the same time, and since then by many workers.

Macy (57) in his review of much of the literature on the quantitative mineral nutrient requirements of plants, concluded from the analyses made on dried plant material that "The central concept of plant analysis is the *critical percentage* of each nutrient in each kind of a plant, above which there is luxury consumption, and below which there is *poverty adjustment* which is almost proportional to the deficiency until a minimum percentage is reached." The existence of the three zones of nutrition, in which the *critical concentration* separated the zone of luxury consumption and the zone of poverty adjustment, was shown by plotting the growth response as the abscissa, and the nutrient concentration of the plants (tops or grain) as the ordinate. The transition from zone to zone was considered to be abrupt rather than gradual.

Definition.—The critical nutrient concentration of a plant with respect to growth may be defined either in terms of the nutrient concentration that is just deficient for maximum growth, or that which is just adequate for maximum growth, or as the concentration separating the zone of deficiency from the zone of adequacy. The latter definition is the one given in the summary of the paper by Macy (57), although the second definition is likewise implied in his presentation, and is the one used by Tyner (63) and recently by Wallihan (84). Theoretically, the three definitions emphasize different viewpoints but in actual practice lead to approximately the same critical concentrations, since the critical concentration, as determined experimentally, is not a point but a narrow range of nutrient concentrations, above which the plant is amply supplied with nutrients, and below which the plant is deficient. However, from the experimental viewpoint, it is much easier to determine the nutrient concentration of a plant when that nutrient is just deficient for maximum growth (zone of rapid change in the slope of the curve), rather than when the nutrient concentration of the plant is just sufficient for maximum growth (zone of zero change in the slope of the curve, $dy/dx=0$). From the practical standpoint, stressing when a plant is just deficient in nutrients (8, 20, 22, 54, 61, 89), rather than when it is just sufficient in nutrients, also appears preferable for indicating when fertilizers should be added to the soil.

Determination of the critical nutrient concentration.—Since the keystone for assessing the nutrient status of a crop is the critical nutrient concentration, the methods for its evaluation are of great importance. Ideally, the critical concentration of a nutrient just deficient for maximum growth should be determined by observing a single plant cell or tissue, or even for a specific physiological reaction. In this determination of the critical nutrient concentration, it would be helpful, too, to alter and to maintain plant cells

at a specified nutrient concentration for all or part of the growth period of the plant. Success in the latter direction has been obtained recently by Loustalot, Gilbert & Drosdoff (62), who regulated the nutrient supply of culture solutions so as to have predetermined potassium and nitrogen concentrations in tung leaves at the time of harvest. However, since none of the suggested procedures are convenient or even feasible at this time, alternative

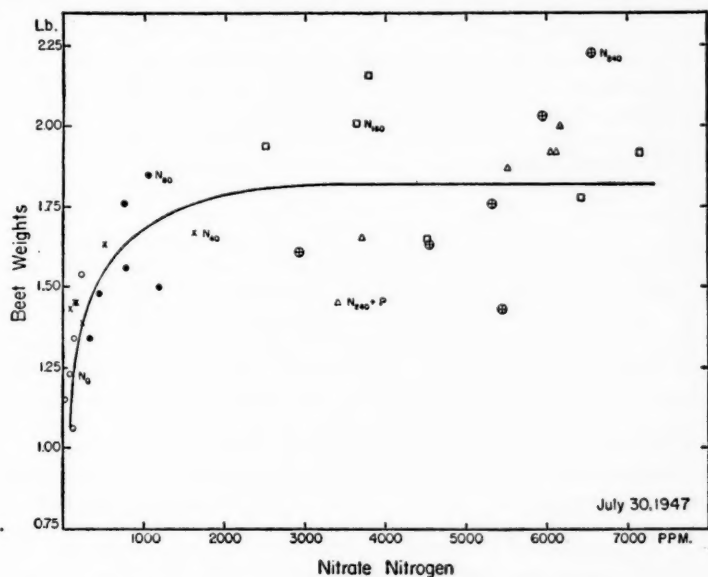


FIG. 3. Relationship of beet root weights (fresh basis) to nitrate-nitrogen concentration (dry basis) of petioles of recently "matured" sugar beet leaves for samples collected from field plots July 30, 1947 (9). The critical nitrate-nitrogen concentration is approximately 1,000 p.p.m. Beets with petioles less than this value tend towards significantly lower beet root weights, whereas those above this value are well supplied with nitrogen and do not differ significantly from one another in weight. The solid line is a free-hand curve drawn to fit the points obtained; the symbols used are defined in Figure 2.

methods must be pursued. The most direct method is through the use of solution or soil cultures or through field experiments, in which a series of plants in pots or field plots are supplied with an increasing amount of the nutrient under study, maintaining all other nutrients at adequate levels (9). In this procedure, the plants are leaf-sampled and harvested when some members of the series are deficient and others are still amply supplied with the nutrients being studied (Figure 3). If this method is not followed, the result may show nutrient differences without yield differences (Figure 4,

June 24), or yield differences without significant nutrient differences (November 5), instead of the desired condition (July 30 and August 28). When the difference in time between sampling and harvesting the crops is considerable, such as in the experiments with corn by Tyner (63), with potatoes by Hill & Cannon (76), and with peas by Tremblay & Bauer (83), it is the reviewer's opinion that the estimation of the critical nutrient concentration is less precise than when sampling and harvesting are done close together. This time factor is particularly important under field conditions, where supplies of nutrients from the soil will not as a rule maintain the concentration of a nutrient at a constant level in the plant for any great length of time. The series of hyperbolic curves relating growth response to nutrient concentration as observed by Burström and discussed by Lundegårdh (12) illustrate some of the difficulties encountered for oats sampled at the time of flowering. They concluded quite correctly that the nitrogen response depended upon the phosphorus level.

The critical nutrient concentration of plants may also be estimated, although somewhat less accurately, by analyzing material from plants showing characteristic deficiency symptoms. Chandler *et al.* (70) have successfully utilized this procedure for potassium deficiency with Ladino clover, Wallace (quoted by Hewitt, 20) for potassium, calcium, and magnesium with apples, and Nicholas (22) for potassium, magnesium, calcium, phosphorus, and nitrogen with cauliflower and potatoes, and for potassium, magnesium, and phosphorus with apples, red currants, and black currants (23). Naturally this approach is only feasible when the symptoms can be recognized readily and when they manifest themselves in a constant manner. For potassium deficiency, the characteristic necrotic spotting on tomato leaves may be replaced by marginal necrosis in the presence of sodium (19), or greatly modified by ammonium (66), while for phosphorus deficiency, the only visible symptom is often a smaller leaf size (55).

Factors affecting the critical concentration.—Although the importance of the relative stability of the critical nutrient concentration from the diagnostic point of view has been recognized, little work has been done to date in this field. Theoretically, the factors that affect the nutrient concentration of a plant could also influence its critical nutrient concentration (61). Macy (57) suggested the possible raising of the critical phosphorus percentage by aluminum, and the possible lowering of the critical potassium percentage by sodium. Ulrich (19, 61) found no large difference in potassium concentration for tomato leaf blades with potassium deficiency symptoms when the plants were grown with or without added sodium in the culture solutions, or in low calcium solutions. At the same time in the presence of sodium the rachis or stem tissues were greatly reduced in potassium concentration, although in neither case did these differences alter appreciably the conclusions regarding the potassium status of the tomato plants. This is especially true when the differences for deficient material are considered in relation to the over-all potassium concentrations of middle rachises, which may range from 0.23 to

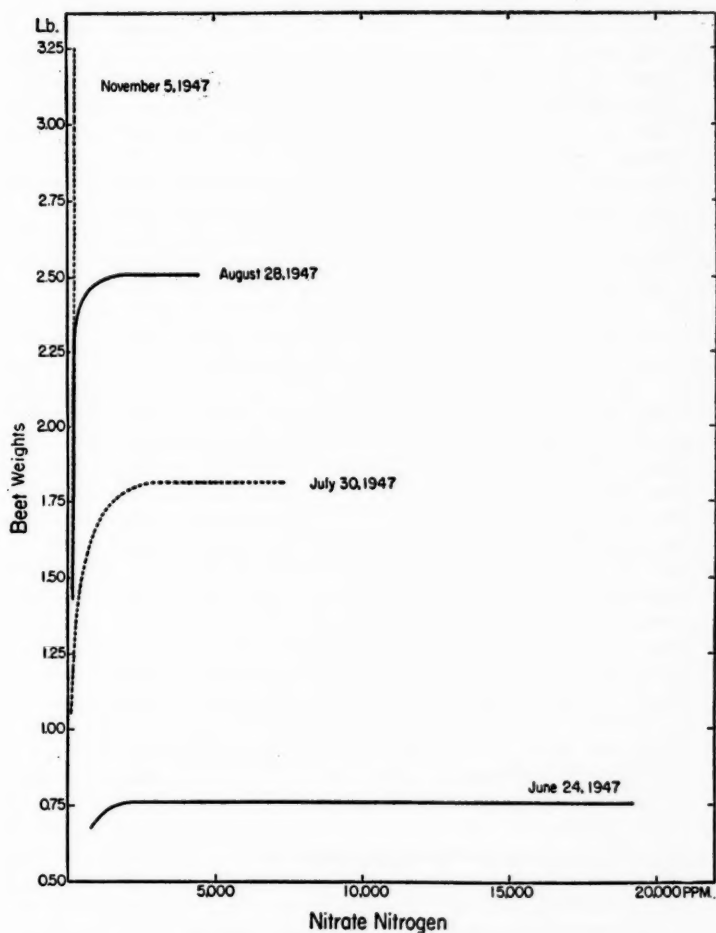


FIG. 4. Gradual transition of beet plants with petioles initially high in nitrate concentration, but not differing significantly in beet root weight (June 24), to beets differing greatly in weight, but with very low nitrate values not differing appreciably from one another (November 5).

Critical nitrate values may be estimated from only the July 30 and August 28 samplings. In both instances the value is approximately 1000 p.p.m. of nitrate-nitrogen.

0.51% for deficient plants, to 10.7% for nondeficient tomato plants (19). During this same experiment, no large differences in potassium concentration of the middle blades and rachises from deficient plants were noted as the plants increased in age. In a review of the results obtained by other investigators (19), it was found that there was no appreciable difference in potassium concentration of leaves with potassium deficiency symptoms taken from plants at different times and places throughout the world. Different species of plants, however, differed considerably in potassium content. For example, varieties of citrus were very low and peach very high (19).

Critical values for nitrogen have been found to be relatively constant for sugar beets when petioles of recently matured leaves were analyzed for nitrate nitrogen. For field-grown beets, values of approximately 1000 p.p.m. were obtained for petioles taken at two different times of the growing season from the same field (Figure 4), while for pot experiments the critical concentration was found to be somewhat less than 3000 p.p.m. (9). For phosphorus the results with sugar beets (56, 82), and with Ladino clover (19) have been consistent for plants grown outdoors in pots and under field conditions. For potassium, Chapman & Brown (8) have concluded from their nutritional studies to date "... that the critical nutrient level for potassium in citrus leaves is constant, and that this value holds irrespective of root-stock or variety." Much study, however, is still needed for all nutrients in order to define adequately the variations in the critical nutrient concentrations which take place under each condition, and by being thus forewarned, misinterpretations of the results from field samples may be prevented to a large extent.

The kind of tissues and the number of tissues included in the plant sample will affect the critical nutrient concentration of a plant. For example, petioles of recently matured sugar beet leaves have a narrower transition zone between deficiency and luxury consumption than the corresponding blades when the plant parts are analyzed for phosphate phosphorus soluble in 2% acetic acid (56). Still broader transition zones would be expected when many tissues are included in the sample, such as when the entire tops of plants are analyzed for their nutrient content, or when immature leaves or growing points are taken as the sample. From both the diagnostic and theoretical viewpoints, a sharp transition zone permits making clearly defined decisions concerning the nutrient status of the plant and in evaluating correctly the factors believed to affect the critical nutrient concentration of a plant.

Nutrient balance and critical concentration concepts.—The nutrient balance concept, as contrasted with that of the critical nutrient concentration concept in evaluating the nutrient status of crops, may appear to be at first glance two opposing viewpoints, but in actuality the balance concept may be considered as an extension of the critical nutrient concept. In the critical nutrient concept, the assumption is made that there is a relatively narrow range of concentrations for a given nutrient at which growth first begins to

decrease. It is further assumed that wide variations, short of excesses in other nutrients, have no large influence on the growth of the plant, and that a major increase in growth is obtained only through the direct or indirect addition of the nutrient in deficiency, although it is recognized that minor increases in growth may be obtained through partial substitutions, as for example, sodium for potassium.

In the nutrient balance concept, the proportion of the nutrients in the dried leaf tissue is considered as well as the actual concentration therein. Initially, Lagatu & Maume (33), and Thomas (15) considered primarily the elements nitrogen, phosphorus, and potassium. They referred to the sum of the percentages N, P_2O_5 , and K_2O , expressed on the dry basis, as the intensity of nutrition and the proportion of these nutrients, calculated as per cent of the total milligram equivalents, as the quality of nutrition. The changes in the quality of nutrition were followed by plotting the values in an equilateral triangle, in which a nutrient assigned to an apex had a value of 100%. While the work of these investigators has aroused a great deal of interest in foliar diagnosis, and many of their concepts concerning technique have been adopted by others, nevertheless, their system of reporting the intensity and quality of nutrition has failed to lead, thus far, to a better understanding of the nutritional status of the plant beyond that given by the far simpler procedure of examining the percentage of each nutrient in the plant in terms of the critical or limiting concentrations. The results with potatoes, reported by Thomas in 1937 (15, Tables I and II), and by Thomas & Mack in 1938 (80, Figure 1), can be interpreted correctly by assuming a critical potash (K_2O) concentration of 2.0% for the potato leaf material. All plants at or below this level for two sampling periods were deficient in potassium as shown by the low yields from these plots.

Recently, Shear *et al.* (64, 65) have extended the concept of nutrient balance and intensity to include all the functional nutrient elements in the leaf. Maximum growth and yield are considered by them to occur only upon the coincidence of optimum intensity and balance. While it appears logical to consider all fifteen elements (carbohydrates, etc., should be included also) as having a specific balance and proper intensity for maximum growth, nevertheless, the practical difficulty of demonstrating the uniqueness of a given balance is not easily attained experimentally. As discussed elsewhere in this review, it is not even possible at present to maintain a single nutrient at a specified concentration within a leaf, let alone a whole series of nutrient elements, for study at given concentrations. Since so little is known of the functions of the nutrient elements within the plant, it would appear to be more expedient to study the less complex relationships of the nutrients to plant growth until more is known of the functions of each nutrient element within the plant.

In contrast to the difficulty of maintaining internal nutrient concentrations at predetermined levels, it is easy to maintain external nutrient solutions at specified values. Experiments have shown that changes in the ex-

ternal nutrient concentrations frequently produce large changes in the internal nutrient concentration of the plant, but plant growth is seldom affected to the same degree. Only at extremes in internal nutrient concentrations is there a significant decrease in the growth of the plant.

CAUSE AND CORRECTION OF DEFICIENCIES

The cause of a deficiency is not given by plant analysis alone, since, for example, a low potassium level in an otherwise satisfactory culture solution will result in an accumulation of calcium and magnesium to high concentrations within the plant whether these two elements are at the regular or increased levels in the nutrient solution. The corrective measure to be applied to a culture solution or a soil low in potassium would be to add more potassium, while for a condition of high calcium or magnesium, it would be to withdraw magnesium and calcium; there would be no indication from leaf analysis alone which alternative to follow. In general, the results of plant analysis tell us what element is deficient, not how the plant became deficient or how to overcome the deficiency effectively. Once the treatments have been applied to the soil, however, their effectiveness can be followed by analyzing plant material taken from treated and untreated plants (Figure 2). An analogous situation would be the interpretation of a single pH determination on a soil. It can neither tell us how the soil became acid or alkaline, or how to change the pH most efficiently.

TIME FACTOR

In interpreting the results of plant analysis, either through the use of the balanced or critical nutrient concentration concepts, the results obtained at any one time apply only to the time of taking the plant samples. When a knowledge of the nutrient status of the plant is required for periods either before or after sampling, additional samples must be taken unless the trends in the nutrient status of the crop have been indicated clearly by correlation or regression studies conducted previously.

The importance of the element of time, in the interpretation of the results of plant analysis, is often overlooked in another way. It takes time for the plant to decrease to a nutrient concentration low enough to decrease its rate of growth, and thereafter, further time is required before the decreases are large enough to be measured experimentally. Thus, when the nutrient concentration of the plant is determined at a definite stage of development, such as the time of flowering (12, 63, 83), or for a given month of the year (17, 54, 61, 72, 73), and the resulting growth correlated with the nutrient content of the plant, the intervening time period from sampling to harvesting is highly important in relation to the supply of nutrients to the plant and the demand made by the plant for these nutrients. Conceivably, plants found deficient in one or more nutrients at the time of sampling could thereafter contain adequate supplies of nutrients either because of an increased supply made available to the plant through rainfall, greater root activity,

etc., or to a lowered demand by the plant for nutrients, which may be brought about by a decrease in growth rate or to a lower requirement for nutrients during the remaining stages in the development of the plant. Under these conditions no growth response would be obtained when one would be expected. In contrast to these findings, plants high in nutrients at the time of sampling could become deficient in one or more nutrients immediately after sampling. Unless additional plant samples are collected and analyzed between the time of determining the nutrient status of the crop and in measuring the growth responses, discordant results may be obtained. Lundegårdh (12), in correlating growth responses with nutrient concentrations of oat leaves at the time of flowering, found that the nitrogen response depended upon the phosphate level of the plant. Smaller nitrogen responses were observed at low phosphate levels, which was interpreted by him as indicating that smaller amounts of nitrogen are required for crops when phosphate levels are low. While this interpretation is correct, it would seem preferable, in order to obtain maximum yields, to add both phosphorus and nitrogen instead of restricting the amount of nitrogen to be added to the soil when phosphorus is low.

RESPONSE

While the results of a plant analysis can indicate readily what nutrient, if any, is deficient at the time of taking the sample, it cannot by itself indicate the amount of response to be obtained by the addition of the deficient nutrient. This depends upon the proximity of the next factor to become limiting, the response being small when a second factor becomes deficient immediately, and large when no deficiency occurs for some time in the growth of the plants.

APPLICATION OF RESULTS

The successful fertilization of any crop in the field is based upon learning: first, the amount of each nutrient required by the crop during a growing season; second, the amount of each nutrient which can be supplied by the soil; and third, the amount of the deficit, if any. The latter is the amount of material to be added to the soil or plant as a fertilizer. Under practical conditions in the field, the major impediment to predicting the amount of nutrients needed by the crop, and the amount to be supplied by the soil, is the lack of an accurate weather forecast. While short term weather predictions have a fair degree of reliability, long range forecasting has not been even mildly successful. In lieu of accurate forecasts, a sample of the seasons, while not giving definitive information for any one year, will still prevent to a large degree over- or under-fertilization of a crop, provided the nutrient status for every crop on a field or cropping unit is also learned each season. Through the accumulation of yearly records through plant analysis, fertilizers will be used more efficiently by simultaneously avoiding excesses and in anticipating deficiencies which may arise for a crop on a field. In this con-

nection, the crop-log system of Clements (11) for sugar cane, or of Nightingale (2, 3) for pineapple, holds great promise for fertilizing the current year's crop in terms of important growth factors (sunlight, temperature, moisture, carbohydrate reserves, etc.), and in providing useful plant nutrient information for subsequent crops in the same field or area.

LITERATURE CITED

1. Emmert, E. M., *Kentucky Agr. Exp. Sta. Bull.*, No. 430, 1-40 (1942)
2. Nightingale, G. T., *Botan. Gaz.*, **103**, 409-56 (1942)
3. Nightingale, G. T., *Botan. Gaz.*, **104**, 191-223 (1942)
4. Clements, H. F., and Kubota, T., *Hawaiian Planters' Record*, **47**, 257-97 (1943)
5. Scarseth, G. D., *Soil Sci.*, **55**, 113-20 (1943)
6. Krantz, B. A., Nelson, W. L., and Burkhart, L. F., in *Diagnostic Techniques for Soils and Crops*, Chap. 5, 137-55 (The American Potash Institute, Washington 6, D. C., 1948)
7. Emmert, E. M., *Proc. Am. Soc. Hort. Sci.*, **54**, 291-98 (1949)
8. Chapman, H. D., and Brown, S. M., *Hilgardia*, **19**, 501-39 (1950)
9. Ulrich, A., *Soil Sci.*, **69**, 291-309 (1950)
10. Ulrich, A., *Proc. Am. Soc. Sugar Beet Technol.*, **6**, 372-89 (1950)
11. Clements, H. F., in *Mineral Nutrition of Plants*, Chap. 18, 451-69 (Truog, E., Ed., The University of Wisconsin Press, Madison, Wisconsin, 469 pp., 1951)
12. Lundegårdh, H., *Leaf Analysis* (English translation by Mitchell, R. L., Hilger and Watts, Ltd., London, England, 176 pp., 1951)
13. McCollam, M. E., *Western Fruit Grower*, **5**(5), 13-16 (1951)
14. Salter, R. M., and Ames, J. W., *J. Am. Soc. Agron.*, **20**, 808-36 (1928)
15. Thomas, W., *Plant Physiol.*, **12**, 571-99 (1937)
16. Thomas, W., *Soil Sci.*, **59**, 353-74 (1945)
17. Lilleland, O., *The Blue Anchor*, **23**, 14 (1946)
18. Goodall, D. W., and Gregory, F. G., *Imp. Bur. Hort. Plantation Crops, East Mallang, Kent, Tech. Commun.*, No. 17, 1-167 (1947)
19. Ulrich, A., in *Diagnostic Techniques for Soils and Crops*, Chap. 6, 157-98 (Kitchen, H. B., Ed., The American Potash Institute, Washington 6, D. C., 1948)
20. Hewitt, E. J., *World Crops*, **2**, 455-59 (1950)
21. Nicholas, D. J. D., *Soils and Fertilizers*, **14**, 191-99 (1951)
22. Nicholas, D. J. D., *J. Hort. Sci. (London)*, **24**, 72-105 (1948)
23. Nicholas, D. J. D., *J. Hort. Sci. (London)*, **24**, 106-22 (1948)
24. Saussure, T. de, *Chemische Untersuchungen über die Vegetation*, **2** (German translation by A. Wieler, Wilhelm Engelmann, Leipzig, Germany, 1890)
25. Liebig, J., *Chemistry in its Application to Agriculture and Physiology* (Playfair, L., and Gregory, W., Eds., John Wiley, New York, N. Y., 1852)
26. Hall, A. D., *J. Agr. Sci.*, **1**, 65-88 (1905)
27. Münter, F., *J. Landw.*, **67**, 229-66 (1919)
28. Gilbert, B. E., and Hardin, L. J., *J. Agr. Research*, **35**, 185-92 (1927)
29. Gilbert, B. E., McLean, F. T., and Adams, W. L., *Plant Physiol.*, **2**, 139-51 (1927)
30. Wolf, B., and Ichisaka, V., *Soil Sci.*, **64**, 227-44 (1947)
31. Emmert, E. M., *Kentucky Agr. Exp. Sta. Circ.*, No. 43 (1934)
32. Pfeiffer, T., Simmermacher, W., and Rippel, A., *J. Landw.*, **67**, 1-57 (1919)

33. Lagatu, H., and Maume, L., *Ann. Ecole Natl. Agr. Montpellier*, **22**, 257-306 (1934)
34. Boynton, D., and Compton, O. C., *Soil Sci.*, **59**, 339-51 (1945)
35. Mitchell, H. L., *Black Rock Forest Bull.*, **5**, 1-138 (1934)
36. Mitchell, H. L., and Chandler, R. F., Jr., *Black Rock Forest Bull.*, No. 11, 1-94 (1939)
37. Hoffer, G. N., *Indiana Agr. Exp. Sta. Bull.*, No. 298, 1-31 (1926)
38. Emmert, E. M., *Plant Physiol.*, **7**, 315-21 (1932)
39. Thornton, S. F., Conner, S. D., and Frazier, R. R., *Purdue Univ. Agr. Exp. Sta. Circ.*, No. 204, 1-16 (1939)
40. Hance, F. E., *Hawaiian Planters' Record*, **45**, 265-96 (1941)
41. Hester, J. B., in *Diagnostic Techniques for Soils and Crops*, Chap. 4 (Kitchen, H. B., Ed., The American Potash Institute, Washington 6, D. C., 308 pp., 1948)
42. Piper, C. S., *J. Agr. Sci.*, **32**, 143-79 (1942)
43. Steenbjerg, F., *Plant and Soil*, **3**, 97-109 (1951)
44. Stout, P. R., and Pearson, G., (Unpublished data)
45. Drake, M., and Scarseth, G. D., *Proc. Soil Sci. Soc. Am.*, **4**, 201-4 (1939)
46. Mitchell, H. L., *Black Rock Forest Papers*, **1**, 30-44 (1936)
47. Arnon, D. I., and Hoagland, D. R., *Botan. Gaz.*, **104**, 576-90 (1943)
48. Ulrich, A., *Proc. Am. Soc. Hort. Sci.*, **41**, 204-12 (1942)
49. Ulrich, A., *Proc. Am. Soc. Hort. Sci.*, **41**, 213-18 (1942)
50. Ulrich, A., *Proc. Am. Soc. Sugar Beet Technol.*, **3**, 66-80 (1942)
51. Brown, R. J., *Soil Sci.*, **56**, 213-22 (1943)
52. Wolff, E., *Aschen-Analysen*, **1** (Wiegandt and Hempel, Berlin, Germany, 194 pp., 1871)
53. Chapman, H. D., *Soil Sci.*, **39**, 111-22 (1935)
54. Chapman, H. D., *Calif. Agr.*, **3** (11), 10, 12, 14 (1949)
55. Ulrich, A., *Proc. Soil Sci. Soc. Am.*, **10**, 150-61 (1945)
56. Ulrich, A., *Proc. Am. Soc. Sugar Beet Technol.*, **5**, 364-77 (1948)
57. Macy, P., *Plant Physiol.*, **11**, 749-64 (1936)
58. Janssen, G., and Bartholomew, R. P., *J. Agr. Research*, **38**, 447-65 (1929)
59. Gilbert, B. E., *Plant Physiol.*, **1**, 191-99 (1926)
60. Lilleland, O., and Brown, J. G., *Proc. Am. Soc. Hort. Sci.*, **36**, 91-98 (1938)
61. Ulrich, A., *Soil Sci.*, **55**, 101-12 (1943)
62. Loustalot, A. J., Gilbert, S. G., and Drosdoff, M., *Plant Physiol.*, **25**, 394-412 (1950)
63. Tyner, E. H., *Proc. Soil Sci. Soc. Am.*, **11**, 317-23 (1946)
64. Shear, C. B., Crane, H. L., and Myers, A. T., *Proc. Am. Soc. Hort. Sci.*, **47**, 239-48 (1946)
65. Shear, C. B., Crane, H. L., and Myers, A. T., *Proc. Am. Soc. Hort. Sci.*, **51**, 319-26 (1948)
66. Wall, M. E., *Soil Sci.*, **49**, 393-409 (1940)
67. Boynton, D., Compton, O. C., and Fisher, E., *Proc. Am. Soc. Hort. Sci.*, **52**, 40-46 (1948)
68. Hamner, K. C., Lyon, C. B., and Hamner, C. L., *Botan. Gaz.*, **103**, 586-616 (1942)
69. Lyon, C. B., Beeson, K. C., and Barrentine, M., *Botan. Gaz.*, **103**, 651-67 (1942)
70. Chandler, R. F., Jr., Peech, M., and Chang, C. W., *J. Am. Soc. Agron.*, **37**, 709-21 (1945)

71. Drosdoff, M., *Soil Sci.*, **70**, 91-98 (1950)
72. Haddock, J. L., *Agronomy J.*, **41**, 79-84 (1949)
73. Haddock, J. L., *Proc. Am. Soc. Sugar Beet Technol.*, **6**, 334-47 (1950)
74. Lucas, R. E., Scarseth, G. D., and Sieling, D. H., *Purdue Univ. Agr. Exp. Sta. Bull.*, No. 468, 1-43 (1942)
75. Terman, G. L., Carpenter, P. N., and Junkins, S. C., *Proc. Soil Sci. Soc. Am.*, **14**, 137-42 (1949)
76. Hill, H., and Cannon, H. B., *Sci. Agr.*, **28**, 185-99 (1948)
77. Lorenz, O. A., *Am. Potato J.*, **21**, 179-92 (1944)
78. Carolus, R. L., *Virginia Truck Exp. Sta. Bull.*, No. 98, 1531-56 (1938)
79. Reuther, W., Smith, P. F., and Specht, A. W., *Proc. Florida State Hort. Soc.*, **62**, 38-45 (1949)
80. Thomas, W., and Mack, W. B., *J. Agr. Research*, **57**, 397-414 (1938)
81. Smith, P. F., and Reuther, W., *Proc. Am. Soc. Hort. Sci.*, **55**, 61-72 (1950)
82. Ulrich, A. (Unpublished data)
83. Tremblay, F. T., and Bauer, K. E., *J. Am. Soc. Agron.*, **40**, 945-59 (1948)
84. Wallihan, E. F. (Unpublished data)
85. Jones, W. W., and Parker, E. R., *Proc. Am. Soc. Hort. Sci.*, **57**, 101-3 (1951)
86. Cain, J. C., and Boynton, D., *Proc. Am. Soc. Hort. Sci.*, **51**, 13-22 (1948)
87. Frear, D. E. H., Anthony, R. D., Haskins, A. L., and Hewetson, F. N., *Proc. Am. Soc. Hort. Sci.*, **44**, 15-24 (1944)
88. Boynton, D., Cain, J. C., and Compton, O. C., *Proc. Am. Soc. Hort. Sci.*, **44**, 15-24 (1944)
89. Ramig, R. E., and Vandecaveye, S. C., *Plant Physiol.*, **25**, 617-29 (1950)
90. Parker, E. R., and Jones, W. W., *Proc. Am. Soc. Hort. Sci.*, **55**, 101-13 (1950)
91. Lynd, J. Q., Turk, L. M., and Cook, R. L., *Agronomy J.*, **42**, 402-7 (1950)
92. Chapman, H. D., and Fullmer, F., *Citrus Leaves* **31** (2), 11, 36-39 (1951)

PHOTOSYNTHESIS¹

BY EUGENE RABINOWITCH

Department of Botany, University of Illinois, Urbana, Illinois

Research in photosynthesis is developing in so many different directions that an attempt to summarize the progress in all of them in this article could amount to not much more than cataloging heterogeneous papers. It seems more useful to review the status of a few important fields. The following have been selected: (a) structure of the photosynthetic apparatus; (b) function of different pigments; and (c) photochemistry of chloroplast preparations and chlorophyll solutions.

STRUCTURE AND COMPOSITION OF THE PHOTOSYNTHETIC APPARATUS

Structure.—It has often been stated that photosynthesis is a "surface reaction." (This conclusion was first drawn from Warburg's finding of inhibition of photosynthesis by surface-blocking compounds, such as urethan.) The meaning of the term "surface reaction" is, however, vague; and its use may be misleading if it makes one think of surfaces of macroscopic (or, at least, microscopic) dimensions. The "surface-blocking" inhibitors may exercise their influence on photosynthesis by attaching themselves to single macromolecules (e.g., protein molecules) rather than by covering surfaces built of many such molecules; on the microscopic scale, photosynthesis may thus be a "three-dimensional" rather than a "two-dimensional" process. Whatever the truth about the last alternative, photosynthesis, in all likelihood, is a topochemical reaction, meaning by this a reaction in which the intermediates are not allowed to move about freely—either in three or in two dimensions—but follow certain paths through the catalytic structure. This channeling of intermediates may provide the solution of the main kinetic puzzle of photosynthesis: How does the cell succeed in preventing the energy-rich intermediates, which must be formed in light, from being lost by back reactions? Quantum yield determinations indicate that losses of this kind are almost completely avoided in weak light, when the rate of formation of the intermediates is slow. They increase as illumination becomes stronger, and ultimately bring about "light saturation"—probably because the "channels" through which the intermediates are carried away, become clogged.

If the surmise that photosynthesis is a "structure-bound" process is correct, then the spatial arrangement of the photocatalytic apparatus must bear a significant relation to the mechanism of photosynthesis.

It was established 17 years ago (1935) by ultraviolet microscopy [Heitz (1), Dautreline (2)] that most if not all chloroplasts consist of a lighter-colored "stroma" impregnated with a number of darker "grana." A typical

¹ The survey of the literature pertaining to this review was concluded in December, 1951.

chloroplast in a mature leaf of a higher plant is shaped like a flat ellipsoid of revolution with a major axis of about 5μ and a minor axis of perhaps 2μ . It may contain from 20 to 100 round, wafer-shaped grana 0.5μ in diameter and 0.1 to 0.2μ in thickness. The dimensions of grana are measured most precisely by electron micrography, with the thickness determined by gold shadow casting. However, the results refer to dry grana, whose thickness, in particular, may be somewhat smaller than that of "live" grana, because of the loss of water and probably also of fluid lipoids during the drying in vacuum. Because of this uncertainty, varying estimates have been made of the fraction of the total volume of a chloroplast occupied by grana in the living state. The average volume of a single dry granum in a mature spinach leaf is of the order of $3 \times 10^{-2} \mu^3$; that of 100 such grana, $3 \mu^3$. This is about 15 per cent of the volume of the live chloroplast (about $20 \mu^3$); *in vivo*, the part of the chloroplast occupied by the grana is larger, but how much larger, we do not know. Frey-Wyssling (3) quoted a much lower figure (only about 6 per cent of the total chloroplast volume occupied by the grana), but this low estimate seems to be incompatible with the large amount of chlorophyll that has to find place in the grana (cf. below).

In algae—as contrasted to leaves—chloroplasts occur in many different shapes and sizes: spiral bands, stars, and amoeboid bodies; but grana, similar to those of the higher plants, have been identified in many (and probably are present in all) of them. However, their size varies from species to species, with the smallest ones falling below the limit of visibility in a light microscope (0.2μ). In algae as well as in higher plants, the size of the grana may change also with the age of the plant and its life history. Grana have been observed also in chloroplast-free blue-green algae [Geitler (4); Vatter (5)], as well as in purple bacteria (cf. below).

It has been suggested that all grana may be located on the surface of the chloroplast; but the best evidence shows that they are dispersed throughout its body, sometimes forming several layers parallelling the large cross-section of the plastid [Heitz (1); Geitler (4)]. Under the influence of certain chemicals, such as acetone, grana have been observed to clump together in one corner of the chloroplast [Weier (6)]; the remainder of the chloroplast then appears colorless, supporting the assumption that all chlorophyll is located in the grana. (This assumption is based primarily on the darker coloration of the grana on ultraviolet micrographs and their fluorescence in the fluorescence microscope.)

The evidence for the occurrence, shape, and structure of grana has been much improved by the application of electron microscopy. The first electron micrographs of chloroplasts have been published by Kausche & Ruska (7) and Roberts (8); since then, much better pictures have been obtained by Granick & Porter (9), Algera *et al.* (10), Frey-Wyssling & Mühlethaler (11), and Vatter (5). The electron micrographs of Wyckoff, described as particularly beautiful by Frey-Wyssling (12), have, to our knowledge, not been published, except for a single one reproduced in Wyckoff's book (13).

Another type of evidence for the existence of small, pigment-bearing particles has been derived by Pardee, Schachman & Stanier (14) from fractional ultracentrifugation of the cell content of purple bacteria, disintegrated by grinding, sonic waves, or sudden release of pressure. All pigments—bacteriochlorophyll as well as the carotenoids—were found to sediment in a single fraction consisting of unusually large particles, which the authors called "chromatophores." (We prefer not to use this name because of its well-established meaning in organic chemistry.) Particles of such large size have not been found in heterotrophic bacteria nor in purple bacteria that had been cultured in darkness and had developed no pigment. (This raises the question whether grana will be found in the chloroplasts of chlorotic higher plants; one may also ask whether the strong quantum-mechanical resonance forces which exist between pigment molecules could be responsible for the cohesion of the grana.)

From the sedimentation constant of the colored fraction of bacteria, Pardee *et al.* calculated—assuming a spherical shape—a particle diameter of 0.04μ . Electron micrographs of the colored fraction revealed the presence of flattened ellipsoids, or round wafers, with a diameter of 0.11μ . The authors suggested that these particles originally were spherical, but became flattened in the preparation of the samples for electron microscopy; on this assumption, a value of 0.06μ was calculated for the diameter of the original spheres, in fair agreement with the above-mentioned value derived from sedimentation experiments (0.04μ). However, agreement can be achieved also by assuming that the bacterial grana have the approximately cylindrical shape observed under the electron microscope—if their thickness is such that in sedimenting in their own plane they would offer a cross-section about equal to that of a 0.04μ sphere. For a diameter of 0.11μ , this means a thickness of 0.05μ . These dimensions are not too far "out of range" to postulate functional identity of the bacterial "chromatophores" with the grana of the higher plants and algae.

Using several somewhat uncertain assumptions, Pardee *et al.* calculated that a single bacterial cell contains about 5000 colored particles; a similar value (6000 grana per cell) was estimated from direct counting of these particles on the electron micrograph.

In a suspension prepared by grinding blue-green algae, fractional ultracentrifugation indicated the presence of chlorophyll-bearing particles of larger size than those found in bacteria; their sedimentation constant was about 300 S as compared to 153 for bacteria. (Electron microscopy indicates the presence in blue-green algae of grana of the usual size—about 0.8μ across.) The large-particle fraction of blue-green algae contained all chlorophyll, but the phycocyanin was present mainly in a slow sedimenting fraction. The occurrence of phycobilin-sensitized chlorophyll fluorescence (cf. section on function of different pigments below) argues against the assumption that this separation of the pigments exists already in the living chromatoplasm, prior to the grinding of the cells.

Starch-free chloroplasts as a whole are known to contain, under normal conditions, 60 to 70 per cent of "proteidic," and 30 to 40 per cent of "lipoidic" material (cf. following section on composition). The "lipoids", i.e., compounds soluble in ether or alcohol, including pigments such as the chlorophylls and the carotenoids—appear to be more-abundant in the grana than in the stroma, but it does not seem that the grana can be purely lipid and the stroma purely proteidic (as one may be tempted to suggest). The high total content of lipoids (up to 35 per cent or even more) in the chloroplasts requires that some of them be present in the stroma as well; while the persistence of grana after treatment with lipophilic solvents indicates that their bodies contain a substantial proteidic "skeleton." The fact that lipophilic stains (such as Sudan Red) color only the grana does not require, according to Frey-Wyssling, that the stroma be lipid-free; rather, this fact can be explained by the assumption that the lipoids in the stroma are associated with proteins to lipoproteids.

Dispersion and fractionation of chloroplastic material has never yet produced a purely lipoidic or a purely proteidic pigment-bearing fraction. The so-called "chloroplastin" of Stoll contained proteins, pigments, and lipophilic materials in approximately the same proportion as whole chloroplasts. Similarly no significant shifts in the [nitrogen]:[chlorophyll] ratio could be observed in the fractionation of dispersed chloroplast material by French, Holt, and others. While these observations make the assumption of protein-free chlorophyll-bearing grana impossible, they do not constitute a proof that chloroplasts contain a stoichiometric compound of chlorophyll and protein, analogous to hemoglobin (as has been repeatedly suggested by different authors).

It was postulated by Hubert (15) and Frey-Wyssling (16) that grana contain alternate proteidic and lipoidic layers, with chlorophyll molecules attached to the former by their chlorophyllin "heads" (made polar by the presence of magnesium and of carbonyl groups) and to the latter by their nonpolar phytol "tails." The "intrinsic" double refraction, exhibited by chloroplasts after imbibition with glycerol (a treatment that does away with the "morphic" double refraction caused by the flat shape of the chloroplasts and grana), has been interpreted [Menke (17); Frey-Wyssling (16)] as evidence of the presence in the chloroplasts of arrays of long hydrocarbon chains, such as those present in lipids and phospholipids; however, Frey-Wyssling (3) noted that this double refraction is so weak as to suggest a very imperfect alignment.

In support of the concept of a stoichiometric protein-chlorophyll compound, Takashima (18) described the preparation of a crystalline chlorophyll protein from clover leaves. The procedure consisted in clarifying a suspension of whole and broken chloroplasts with α -picoline (or pyridine), washing it by dialysis through cellophane into a 50 to 55 per cent aqueous solution of the same organic base, and filtering the green solution remaining in the cellophane bag from the carotenoid crystals formed in it. The peak of the

red absorption band of the filtrate was at 668 $m\mu$, as compared to 678 $m\mu$ in the original suspension. Addition of dioxane until the solution contained 20 per cent dioxane, 43 per cent x-picoline (or pyridine) and 37 per cent phosphate buffer (pH 7.0) led—after standing for 4 to 7 days in the icebox—to the precipitation of clusters of needle-like green crystals. The presence of protein in these crystals was confirmed by nindhydrin, xanthoproteid, and biuret reactions; its molecular weight (in 55 per cent picoline) was estimated osmotically as about 19000. It contained 0.61 per cent P, indicating the possible presence of phospholipids. Spectrophotometric estimates indicated the presence of between 1.3 and 2.4 molecules of chlorophyll per protein unit of 19000.

Electron micrographs of chloroplasts show, beside the flat cylindrical grana, various still thinner structures whose nature is as yet uncertain. Frey-Wyssling (3) distinguishes three types of them. Some are large, irregular in shape, and creased in a way suggestive of burst membrane bags, the contents of which have leaked out. Often they are so large as to indicate that they must have enclosed a whole chloroplast. Single grana are often seen left in the bag, and in two photographs [one by Frey-Wyssling & Mühlethaler (11) and one by Wyckoff (13)], the bag still encloses what looks like a full complement of grana present in a chloroplast; partial leaching-out of the stroma can explain its numerous folds. It remains to be proved, however, that this membrane is not an artefact, particularly since similar folded bags of smaller size can also be found on many micrographs. A second type of thin, flat objects, also regularly appearing on the electron micrographs, are uniform (noncreased) and often circular or oval in shape; sometimes they appear finely granular. These round specks are interpreted by Frey-Wyssling as deposits left after the drying-out of drops of lipoids that had leaked out of grana (or the stroma) during the preparation (either by dissolution in lipid solvents—if these have been used in washing—or through melting). Sometimes these thin pancakes lie isolated on the supporting collodion film; sometimes they protrude from the grana—like ham slices from a bun—as if they had dried out while the lipid was in the process of leaking out of the granum. In some cases, these protuberances are angular and streaky (rather than round and smooth); this changed appearance might be a result of quantitative differences in surface tension rather than of qualitative differences in composition. All these artefacts probably are similar in nature to the so-called "myelin tubes," formed by lecithin and other phospholipids in contact with water. Such growths have been observed before under the light microscope, in chloroplasts exposed to aqueous detergents or alkalis.

Another kind of thin lamellae, also observed in chloroplast preparations under an electron microscope is particularly interesting because it does not appear to be an artefact. Some micrographs by Frey-Wyssling & Mühlethaler (11), and particularly, by Vatter (5), show groups of up to 25 thin circular discs, uniform in size and looking like a scattered roll of coins; their

diameter is about equal to that of a granum, and their thickness only about $5 \times 10^{-4} \mu$ (50 Å). It appears as if a single granum has disintegrated into one or two dozen of such discs. The assumption that they are normal structural elements of a granum seems plausible in the light of the above-mentioned—admittedly speculative—picture of grana as consisting of alternate layers of proteidic and lipidic material. Since these “money rolls” can be observed also in preparations which have been treated with lipophilic solvents, one can imagine that they are protein discs which became separated when the lipid “glue” that held them together, leaked out of the granum. The thick wafer-shaped grana, as they appear on electron micrographs, are stacks of such discs, held together by lipid material interlarded between them.

In addition to these three types of thin flat structures, electron micrographs of chloroplastic material show a scattering of small but uniform spherical grains, about 250 Å in diameter, often covering the whole background of the micrograph. It has been suggested that these are globular proteins, probably originating in the stroma and left scattered after the lipid moiety of the lipoproteids to which they belonged, has been dissolved (or melted) during the preparation of the slide. The molecular weight of these micromolecules is of the order of 5×10^6 .

We do not know which among all the above-mentioned forms contain chlorophyll (and the other photosynthetically important pigments); perhaps this problem could be approached by photochemical experiments (e.g., chlorophyll-sensitized precipitation of silver from silver nitrate solution). Frey-Wyssling (3, 11) suggested that chlorophyll is most closely associated with the protein discs in grana; but he also mentioned the possibility that chlorophyll may leak out of the grana with the lipoids.

Fully green chloroplasts contain, on the average, 10 to 15 per cent chlorophyll and other pigments (in relation to dry weight). This corresponds to about 1 to 2×10^9 pigment molecules per chloroplast, or average pigment concentration of the order of 0.05 to 0.1 mole/l. in the chloroplast as a whole.

In a single granum, the number of pigment molecules should be of the order of 1 to 2×10^7 ; chlorophyll concentration should thus reach 0.2 to 0.4 mole/l. Grana probably are considerably denser and contain much less water than the predominantly proteidic phases of the cell (such as the stroma and the cytoplasm); nevertheless, the proportion of chlorophyll in the dry matter of the grana must be very high—perhaps 20 to 30 per cent.

If one considers a granum as a disc 0.5μ in diameter, its two large surfaces have a total area of $0.4 \mu^2$. According to surface film measurements of Hanson (19) chlorophyll molecules, stacked like books on a half-filled shelf, require an area of about $1 \mu^2$ apiece. That means that not more than 4×10^5 chlorophyll molecules could find place in the two surface layers of the granum, and that about 25 complete layers of chlorophyll must be present in each of them. The thin discs into which grana have been observed to disintegrate might thus contain one or two layers of chlorophyll molecules each—perhaps one layer on each side of the disc.

With an average chlorophyll concentration of 0.2 to 0.4 mole/l., the average distance between centers of two chlorophyll molecules would be of the order of 15 to 20 Å if these molecules were distributed at random in the granum. The average distance from a molecule of a carotenoid, or a phytyl-bilin molecule imbedded in this structure, to the nearest chlorophyll molecule would be of the same order of magnitude. However, because of the probable arrangement of the chlorophyll molecules in layers, the distance between the centers of two neighboring chlorophyll molecules may be considerably shorter, perhaps as short as 5 Å (which is the probable "thickness" of the porphyrin ring system).

Composition.—Not much is known about the chemical nature of constituents of the chloroplasts other than the pigments, except for their above-mentioned general division into "proteidic" and "lipophilic" compounds. Earlier data on the amount and properties of proteins and lipoids in chloroplasts were summarized elsewhere (20, Chapter 12). Since then, new studies have been made, e.g. by Timm (21), Sisakyan, Bezinger & Kuvaeva (22) and Osipova & Timofeeva (23). Sisakyan and co-workers have used paper chromatography to identify 16 amino acids in chloroplast proteins. Osipova & Timofeeva found that chloroplast proteins reduce more ferricyanide than cytoplasmic proteins, and that their capacity to adsorb chlorophyll from alcoholic solution parallels their reducing capacity. After oxidation with ferricyanide, chloroplast proteins lost about three-fourths of this capacity and their reducing power became the same as that of proteins from the cytoplasm. When chloroplast proteins adsorb chlorophyll, their capacity to be oxidized by ferricyanide is largely lost, indicating that chlorophyll blocks their reducing groups.

Osipova & Timofeeva (24) observed the changes in chloroplast composition caused by nitrogen deficiency and by darkness. With one fourth of the normal nitrogen supply, protein concentration in chloroplasts went down from 70 per cent to 26 per cent, while the amount of starch rose from 3 to 30 per cent.

Chloroplast ash was investigated by Neish (25), Hill & Lehmann (26), Noack & Liebich (27), and by Veher (28). Neish found Mg, Cu, Fe, Ca, P, K, Na, Mn, S, and Cl; Veher added to this list Co and Mo. The presence of heavy metals and phosphorus probably indicates high enzyme content.

According to Neish (25) and to Menke (29), two or three times as much phosphorus is present in the chloroplasts as in the cytoplasm; but the chloroplasts contain less Ca and Mg (despite the presence of chlorophyll), and very little K.

From the point of view of photosynthesis, one is most interested in the occurrence in chloroplasts of enzymes whose action could possibly be related to the fixation and reduction of carbon dioxide and the liberation of oxygen.

Among the carbon dioxide binding enzymes, one first inquires about carbonic anhydrase. It has been suggested that the hydration of carbon dioxide to H_2CO_3 could be a "bottleneck" in photosynthesis [cf. (20), p.

198]. Carbonic anhydrase could not be found in leaves by Burr (30) and Mommaerts (31); but Neish (32), Steeman-Nielsen & Kristiansen (33), Day & Franklin (34), Bradfield (35), and Waygood & Clendenning (36) found that chloroplast material has a definite catalytic effect on the hydration of carbon dioxide.

It is generally assumed that carbon dioxide enters photosynthesis through carboxylation of an organic molecule. Vennesland and co-workers (37) have identified a number of carboxylases in plant material, using mostly nonchlorophyllous tissues. Waygood & Clendenning (36) found that in leaves all known carboxylases (pyruvic, oxalacetic, oxalsuccinic, ketoglutaric, and glutamic) were present in cytoplasmic rather than in chloroplastic material.

A special position among carboxylases belongs to the so-called "malic enzyme" discovered by Ochoa and co-workers in animal tissues, but found also in plant tissues by Vennesland and co-workers (37). This enzyme catalyzes the transformation of pyruvate into malate—a combination of decarboxylation and dehydrogenation, with triphosphopyridine nucleotide (TPN) as hydrogen acceptor. Arnon (38) found malic enzyme in sugar beet leaf mash from which chloroplasts had been removed so that it is probably located in the cytoplasm.

There have been indications in C^{14} experiments by Frenkel (39) that the primary carboxylation in photosynthesis takes place in the cytoplasm rather than in the chloroplasts; but these experiments are in need of repetition, taking into consideration the since-established, nonphotosynthetic exchange uptake of radiocarbon by plant tissues. If reductive carboxylation were established as the first step of photosynthesis, its localization in the cytoplasm would have an added meaning, since it would necessitate the diffusion of a photochemically produced reductant from the chloroplast into the cytoplasm. (The question whether the primary photochemical process produces a comparatively stable intermediate of strong reducing power is one of the presently most controversial problems in photosynthesis.)

Boichenko (39a) asserted that clover leaf chloroplasts contain an enzyme (hydrogen lyase?) capable of combining carbon dioxide and hydrogen to formic acid; but this certainly needs confirmation.

Coming now to enzymes engaged in the transfer of hydrogen from organic substrates to oxygen (which conceivably could also be useful in the reverse process), we note that cytochrome-*c* was repeatedly reported in plants, but first successfully extracted from leaves by Hill & Scarisbrick (40). Cytochrome oxidase which mediates between oxygen and cytochrome-*c*, was reported in isolated spinach chloroplasts by Rosenberg & Ducet (41). Hill & Scarisbrick (40) found cytochrome-*c* to be distributed in both chlorophyllous and nonchlorophyllous plant tissues. They also discovered two new plant hemoporphyrins, which they designated as cytochrome-*b₃* and cytochrome-*f*, the second of which they found to be present in chlorophyllous tissues only—not alone in leaves but also in the few species of algae which were tested. They believed it to be associated with the chloroplasts. One

molecule of cytochrome-*f* was present per about 400 molecules chlorophyll. Cytochrome-*f* was found by Davenport & Hill (42) to have a more positive potential than cytochrome-*c* ($E_0 = +0.365$ vs $+0.260$ v.). No cytochrome-*f* oxidase has yet been found.

An alternative mechanism of hydrogen transfer to oxygen in respiration involves polyphenol oxidase (=tyrosinase or catecholase) instead of a cytochrome oxidase. This oxidase is widely found in plants, although Holt (43) found no evidence of it in *Phytolacca americana*.

Arnon (44) reported that in beet leaves, the polyphenol oxidase is localized in the chloroplasts; Warburg & Lüttgens (45) and Bonner & Wildman (46), on the other hand, noted that most of the oxygen uptake by mashed spinach leaves—which is attributable to the activity of a polyphenol oxidase—took place in the cytoplasmic fluid, and not in the chloroplastic material.

According to Neish (32) all catalase of green plants is present in chloroplasts; but Krossing (47) found it in the cytoplasm as well.

No pyridine nucleotides, di- or triphosphopyridine nucleotide (DPN or TPN) have yet been identified in chloroplast material. Conn and co-workers (48) have proved the presence in different plant tissues of enzymes that rapidly decompose pyridine nucleotides; it is therefore possible that DPN or TPN are present in chloroplasts but are destroyed during extraction.

Since starch grains are formed and dissolved in chloroplasts, one expects to find in them enzymes engaged in hydrolysis and mutual transformation of carbohydrates, such as amylase, invertase, etc. [cf. Sisakyan & Kobjakova (49); Nezgovorov (50)]. Proteases, too, have been identified in chloroplasts [Sisakyan & Kobjakova (49)].

FUNCTION OF DIFFERENT PIGMENTS

The chloroplasts of green plants, chromoplasts of colored algae, and the chromoplasm of the blue-green algae, all contain a mixture of pigments of various chemical character. The three main types are the chlorophylls (green), the carotenoids (yellow), and the phycobilins (red or blue).

Engelmann first suggested as early as 1882 that light energy absorbed by all these pigments is utilizable for photosynthesis. However, after the publication of Willstätter & Stoll's fundamental monographs on chlorophyll and photosynthesis (in 1913 and 1918, respectively), it has become customary to consider chlorophyll as the photosynthetic pigment *par excellence*. In addition to a single (and unconvincing) experiment of Willstätter & Stoll, this belief was based only on general occurrence of chlorophyll (more specifically, chlorophyll *a*) in all photosynthesizing organisms (except purple and green bacteria, which, however, contain analogous pigments—"bacteriochlorophyll" and "bacterioviridin," respectively).

In 1930 to 1950, many experiments by Emerson, Manning, Blinks, and others have completely vindicated Engelmann's hypothesis, by showing that all three types of pigments can contribute energy to photosynthesis.

However, in some organisms, certain pigments are less effective than others. Thus, the carotenoids of green algae appear to be about one-half as efficient as chlorophyll [Emerson & Lewis, (51)], while in blue-green algae, they are only 20 per cent as efficient [Emerson & Lewis (52)]; the principal carotenoid of brown algae, fucoxanthol, on the other hand, seems to have the same efficiency as chlorophyll [Dutton & Manning (53); Tanada (54)]. In certain species of red algae (if not all of them), light absorbed by chlorophyll is used for photosynthesis less efficiently than light absorbed by phycoerythrin [Haxo & Blinks (55)]. Recent experiments in Blinks' laboratory (56) showed that the sensitizing efficiency of chlorophyll in red algae depends on previous history of the cells: pre-illumination with red light promotes it, while pre-illumination with green light destroys it.

In purple bacteria [Thomas (57)], light quanta absorbed by bacteriochlorophyll and by several carotenoids contributes to photosynthesis, but not those absorbed by the main carotenoid present in these organisms, spirilloxanthin.

Despite the now established fact that pigments other than chlorophyll often are equal (and in some cases even superior) to chlorophyll as suppliers of energy for the photosynthetic process, the concept of chlorophyll as the primary photosynthetic pigment has not been discarded. It has even received new experimental support. This support is derived from the observation that in photosynthesizing cell organs, pigments are packed so densely that light energy absorbed by any one of them can be transferred to another one, provided it has a suitably located excitation level. This transfer is revealed by emission of "sensitized fluorescence." Thus, in diatoms, absorption of light by fucoxanthol produces fluorescence of chlorophyll with the same quantum efficiency as absorption by chlorophyll itself. [Dutton, Manning & Duggar (58)]. In *Chorella*, too, chlorophyll fluorescence can be excited by light absorbed by carotenoids, but, in this case, with a lower efficiency than by light absorbed by chlorophyll itself [Vermeulen, Wassink & Reman (59).] (It was noted above that in green algae the sensitization of photosynthesis by carotenoids also is less efficient than that by chlorophyll.) In red algae, excitation energy transfer can be observed from carotenoids to the phycobilins, as well as from the phycobilins to chlorophyll; within a single group of pigments, energy transfer has been observed from phycocyanin to phycoerythrin, and from chlorophyll *a* to an unknown pigment [probably "chlorophyll d," discovered in algae of this class by Manning & Strain (60)], whose absorption band lies on the long-wave side of that of chlorophyll *a* [Duysens (61); French & Young (62)]. Similarly, evidence has been obtained (*in vitro*) of excitation transfer from chlorophyll *b* to chlorophyll *a* [Duysens (61); Livingston (63)]. In purple bacteria, energy transfer (which is shown by sensitized fluorescence) occurs from all carotenoids, except spirilloxanthin (again, we note the analogy to photochemical behavior!), to bacteriochlorophyll, and, among the several (apparently three) bacteriochlorophylls present in these organisms, to the one whose absorption band has the

lowest frequency [Duysens (61)]. In every case, it thus appears, excitation quanta migrate through the pigment-bearing structure until they are "trapped" in the pigment with the lowest excitation level (i.e., the pigment whose absorption band lies furthest in the red). This pigment is chlorophyll *a* in all plants, except those in which chlorophyll *d* is present. The excitation energy remains "trapped" in the last pigment long enough for some of its quanta to be re-emitted as fluorescence, and is then dissipated, by conversion into heat or chemical energy, with or without the intermediary of a metastable electronic state.

In red algae, a peculiar phenomenon was found by Duysens (61) as well as by French & Young (62): phycoerythrin-sensitized fluorescence of chlorophyll *a* proved to be stronger than its directly excited fluorescence. As mentioned above, direct excitation of chlorophyll *a* apparently leads, in these algae, to energy transfer to another pigment, present in very small quantity and having an absorption band at about 730 $m\mu$ —probably, chlorophyll *d*; this pigment fluoresces quite strongly in the light absorbed by the (much more abundant) chlorophyll *a*. This observation makes one think of Haxo & Blinks' finding of relative photosynthetic ineffectiveness of chlorophyll *a* in red algae. It appears as if in these algae a large part of chlorophyll *a* is rendered nonfluorescent and photosynthetically inactive by association with chlorophyll *d*; the latter functions as a "sink" into which the energy absorbed by chlorophyll *a* disappears. However, enough chlorophyll *a* must remain free from this fatal association to permit effective sensitization of photosynthesis (and emission of the fluorescence band of chlorophyll *a*) by the energy quanta first absorbed by the phycobilins. In other words, these algae seem to contain two pigment complexes—one containing the phycobilins and a small amount of chlorophyll *a*, and the other containing most of the chlorophyll *a* together with a small amount of chlorophyll *d*. The "uncoupling" of the bulk of "d-contaminated" chlorophyll *a* from the photosynthetically active carotene-phycobilin-chlorophyll *a* complex seems to be induced by pre-illumination with green light (absorbed mainly by the phycobilins) and reversed by pre-illumination with red light (absorbed mainly by chlorophyll).

The reasons why light energy flowing into chlorophyll *d* is lost for photosynthesis must remain a subject of speculation until the chemical nature and photochemical properties of this pigment are better known. It may be that the quantum of excitation energy available in chlorophyll *d* is just a trifle too small to sensitize photosynthesis (the absorption band of chlorophyll *d* is located, *in vivo*, at 730 $m\mu$, as against 670 $m\mu$ for chlorophyll *a*). It may also be that the low concentration of chlorophyll *d* (apparently, under 0.1 per cent of chlorophyll *a* in the algae studied by Duysens) makes the trapping of the majority of absorbed excitation quanta in this pigment unfavorable by causing "light saturation" to occur in very weak light. (Only a very small amount of a rate-limiting enzyme may be available in sufficient proximity to the few chlorophyll *d* molecules.) As a third alternative, trap-

ping of energy quanta in a few isolated chlorophyll *d* molecules may prevent these quanta from moving, by resonance, to the place where they are needed for the photochemical reaction (e.g., to the surface of a granum).

These observations favor the concept that the usual—if not the only—path of energy utilization in photosynthesis is via chlorophyll *a* in its lowest excitation state—i.e., the upper level of its red absorption band, with an electronic energy content of approximately 42 kcal./mole. If the primary absorption is by a different pigment, the excitation quantum is transferred to chlorophyll *a* by "resonance migration"; if the absorption is in the blue-violet band of chlorophyll *a*, the electronic energy difference between the "blue" and the "red" excitation level (about 21 kcal./mole) is dissipated into heat—probably via conversion into intermolecular vibrational energy—and the chlorophyll molecules pass into the same excited state as after absorption of red light. This is revealed by the emission of red fluorescence by chlorophyll excited by blue or violet light. New measurements by Livingston and co-workers [superseding those quoted earlier (20, chapter 23)] re-established the truth of the original assumption that the quantum yield of red chlorophyll fluorescence *in vivo* is the same whether primary excitation had occurred in the blue-violet, or the red band. Since blue fluorescence has never been observed in chlorophyll (*in vitro* or *in vivo*), the transformation must be very fast—it must be completed in much less than 10^{-11} sec. (If the excited state of the chlorophyll molecule, reached by absorption in the blue-violet band, would survive for 10^{-11} sec., blue fluorescence would have to appear with a quantum yield of about 0.1 per cent, and would thus be easily recognizable.) It is possible—but by no means certain—that before a "red" excitation quantum of chlorophyll *a* is used for photosynthesis (or another photochemical reaction), the chlorophyll molecule undergoes further transformation, with the loss of an unknown fraction of its excitation energy—this time into a nonfluorescent metastable state (a "triplet" state, characterized by two unpaired electron spins, i.e., two non-saturated valencies either at the same or at two different carbon atoms). However, this conclusion is based on analogy with other organic pigments more than on specific evidence obtained in the study of chlorophyll. The only supporting argument for the existence of metastable, energy-rich chlorophyll molecules is derived from the observation that a high quantum yield of certain chlorophyll-sensitized autooxidations in solution (e.g., of the autooxidation of thiourea) can be maintained at very low concentrations of both the oxidation substrate and oxygen. The assumption of a long-lived active state of chlorophyll seems to be needed in this case to account for the efficient energy transfer from the light-excited pigment to one of the two reactants. However, an alternative explanation is feasible if one assumes that complex formation takes place in the dark between the sensitizer and one of the reactants, eliminating the necessity for an excited molecule of the sensitizer to survive until it meets this reactant in a kinetic encounter.

If the assumption of a metastable chlorophyll molecule as intermediate energy carrier in photosynthesis is correct, the conversion into the meta-

stable state must occur within less than 10^{-10} sec. after the original excitation, to account for the fact that the red chlorophyll fluorescence *in vivo* has a quantum yield of the order of 0.1 per cent to 0.5 per cent. [The "natural" lifetime of an excited chlorophyll molecule—i.e. the time after which all excitation would have been lost by fluorescence—is, according to Livingston (63), approximately 1×10^{-8} sec.; for the fluorescence yield to be reduced to 5×10^{-3} , the lifetime of the excited state must be shortened proportionally, i.e., to 5×10^{-11} sec.] If the transfer into the metastable state is the normal fate of an excited chlorophyll molecule, then the event that prematurely terminates the fluorescent state must be this transfer, rather than the complete conversion of electronic excitation energy into vibrational energy (and thence into heat).

In this picture, the quantum yield of photosynthesis in light of different wavelengths is the measure of the relative efficiency with which the light quanta, absorbed in this spectral region by the several components of the pigment mixture, are transferred to chlorophyll *a* and stored there as the smallest available electronic excitation quanta of this pigment.

One phenomenon which has not received satisfactory explanation is the rapid decline of the quantum yield of photosynthesis [Emerson & Lewis (64)], as well as of the Hill reaction [Ehrmantraut & Rabinowitch (65)] and of fluorescence [Livingston (66)] that occurs when the wavelength of the exciting light exceeds $670 \text{ m}\mu$, i.e., lies on the "red" side of the peak of the red absorption band. (A similar drop of fluorescence yield has been noted in solutions of other dyestuffs.) Vavilov (67) suggested a thermodynamic explanation of this drop, but a thermodynamical theory should always be translatable into the language of molecular statistics, and this has not yet been possible in this case.

Additional evidence confirming the more intimate association of chlorophyll *a* with photosynthesis (compared to the phycobilins) can be derived from the observation by French *et al.* (62) that the fluorescence of the phycobilins does not show, during the induction period of photosynthesis, the characteristic changes in intensity that chlorophyll fluorescence exhibits according to the measurements by Kautsky, McAllister, Franck, and their co-workers. The intensity of the red chlorophyll fluorescence fluctuates in the first seconds and minutes of illumination in a way clearly related to the simultaneous fluctuations of the rate of oxygen liberation.

"Resonance migration" of excitation energy between pigment molecules is a phenomenon to be expected both in classical electromagnetic theory and from the quantum-mechanical point of view. J. Perrin (68) and F. Perrin (68), Vavilov & Feofilov (69), and Arnold & Oppenheimer (70) made calculations of the probability of such energy transfer as the function of distance between the excited and the nonexcited molecule; the estimates of Arnold & Oppenheimer were specifically intended to evaluate the likelihood of such a transfer from phycoerythrin to chlorophyll at the pigment concentrations existing in the chloroplasts of red algae.

The most adequate theoretical treatment of resonance transfer to date

is that by Förster (71). The results indicate that the probability of the transfer must decrease with the sixth power of the distance between the "energy donor" and the "energy-acceptor," and is the higher the greater the overlapping of the absorption band of the "acceptor" with the fluorescence band of the donor. (This overlapping is a measure of the "resonance" of the two pigments.) In chlorophyll—where the fluorescence band lies much closer to the absorption band than in most other pigments, with the peaks of the two bands being separated by only 5 to 10 $m\mu$ (cf. 20, Table 23.1) the probability of energy transfer must be particularly high. According to Förster, it should reach 50 per cent at a distance of about 80 Å between the two energy-exchanging molecules, assuming that the excited state survives its full natural lifetime. It was estimated in the section on the structure of the photosynthetic apparatus of this review that the average distance between two chlorophyll molecules in a granum is less than 20 Å; this should be enough, according to Förster, for many thousands of transfers, and could thus lead to a complicated "Brownian movement" of the excitation energy through the granum. However, as pointed out by Franck & Teller (72), but not considered by Förster, the migration path is reduced, in the living cell, by a factor of the order of 10^3 , because excited chlorophyll molecules survive for only a few thousandths of their natural excitation time. (They may then pass into a metastable state, which lives much longer, but however energy-rich this state may be, it does not allow an effective energy migration). It thus appears that the possibilities of energy migration between pigment molecules *in vivo* are closely restricted—perhaps to a few shifts between adjoining molecules only. This may well be sufficient to transfer most if not all excitation quanta from the carotenoids (or the phycobilins) to chlorophyll, as well as to convey the energy quanta from the chlorophyll molecules in the center of a granum to the surface of the latter, which may be the only place where the energy quanta have a chance to meet the substrate molecules and to initiate photosynthesis. On the other hand, this migration path is not sufficient to justify the concept of a "photosynthetic unit," proposed by Gaffron & Wohl (73) to explain certain kinetic properties of photosynthesis: This picture required a quantum to be available, during the excitation period, in any one of a group of about 2000 chlorophyll molecules. Förster, who neglected to take into account the reduced lifetime of chlorophyll excitation *in vivo* thought that his calculation of the transfer probability proves the "photosynthetic unit" to be physically possible.

It should be noted that in the kind of "Brownian movement" of excitation energy discussed by Förster, the electronic excitation dwells long enough in each visited molecule to get into equilibrium with the intermolecular vibrations of this molecule (since a "visiting time" of 10^{-11} sec. still is long compared to the period of a single molecular vibration, which is of the order of 10^{-13} sec.). Therefore, the position and shape of the absorption band of the pigment, determined as it is by the interaction of electronic excitation

and molecular vibrations, is not strongly affected by the migration of electronic energy; one can say that the absorption act remains localized in an individual pigment molecule, even if the excitation energy does not stay in this molecule. With closer coupling, however, electronic energy exchange can become so much faster that the electronic excitation will sweep over the individual molecules leaving their vibrational states unchanged. The excitation will then cease to be localizable; and the excited state will belong to the pigment system as a whole, rather than to an individual pigment molecule. The absorption band must be deeply affected by this type of "fast" energy exchange, since a whole system of pigment molecules now acts—in the excited state—like a single, giant molecule.

Förster showed that, under certain conditions, the spectral effect of such fast excitation exchange will be similar to that actually observed by Scheibe (74) in micelles which were formed by the reversible polymerization of certain dyestuffs in solution. The rather broad absorption band of the monomeric form of such a dyestuff is replaced, upon polymerization, by a new and very sharp band, displaced by as much as 50 to 60 $m\mu$ (~ 3000 cm^{-1}) towards the red from the original band. The sharpness of the band indicates the absence of strong coupling between electronic excitation and vibrations; this "protected" character of the electronic excitation finds another expression in a strong resonance fluorescence, observed in the micelle band—a type of fluorescence very uncommon in complex organic molecules. (Loss of electronic excitation energy by conversion into vibrations during the excitation period usually causes, in such molecules, a displacement of the fluorescence band to the red from the absorption band, as expressed in so-called Stokes' rule.)

The absorption spectrum of chlorophyll in living cells is characterized by a "red shift" of all absorption bands by 300 to 400 cm^{-1} from their position in organic solution (from 660 to about 675 $m\mu$), without much change in the shape of the absorption bands. This relatively minor change indicates that chlorophyll in the chloroplasts does not form a resonating system of the type present in Scheibe's dyestuff polymers. Förster pointed out that this spectroscopic picture is not incompatible with "slow" resonance transfer of excitation energy—the type in which the energy stays with a single molecule longer than 10^{-12} sec., thus permitting up to 10^4 transfers during an excitation time of 10^{-8} sec. (the full natural lifetime of excited chlorophyll molecules), but fewer than 100 transfers in the 10^{-10} sec. actually available to excited chlorophyll *in vivo*.

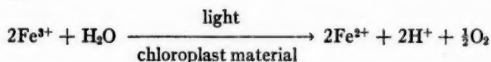
Jacobs (75) noted that colloidal particles of ethyl chlorophyllide (formed by addition of water to methanolic or acetonetic chlorophyllide solution) have an absorption band at 735 $m\mu$ shifted fully 75 $m\mu$ from its position in molecular solution (660 $m\mu$). These particles are revealed by electron microscopy to be crystalline. (They appear as triangular plates with sharp angles.) Similarly prepared colloidal chlorophyll (with intact phytol chain) has an absorption band at about 670 $m\mu$ —only 10 $m\mu$ from its position in

molecular solution. These colloidal particles appear noncrystalline under the electron microscope. (It is well known to chemists that chlorophyll does not crystallize!) In the formation of colloidal ethyl chlorophyllide, similar noncrystalline aggregates, with an absorption peak at 670 $m\mu$, are formed within 0.1 sec. after the mixing of the solvents; they are then converted, at a measurable rate, into the above-mentioned crystalline particles with an absorption peak at 735 $m\mu$. A spectroscopic change of the type indicative of the initiation of "fast" excitation exchange thus seems to occur only upon a truly crystalline arrangement of chlorophyll chromophores, but not upon a more or less disorderly aggregation of pigment molecules in a noncrystalline colloidal particle.

To sum up, resonance interaction of photosynthetic pigments in living cells seems to be of a strength that makes possible the migration of excitation energy through a small number of pigment molecules. This can account for the observed sensitized fluorescence of chlorophyll *in vivo*, and makes feasible a two-step mechanism of sensitization of photosynthesis, in which one kind of pigment molecule absorbs the quanta and transfers them to another kind which interacts with the substrate. A similar energy transfer must be possible also between identical molecules—e.g., adjacent chlorophyll molecules in a granum; but experimental demonstration of this type of transfer is much more difficult than in the case of two different pigments, where sensitized fluorescence serves as a qualitatively and quantitatively reliable index of the energy exchange.

PHOTOCHEMISTRY OF CHLOROPLAST PREPARATION

Hill (76) found that the weak burst of oxygen that Friedel had noted in 1901 upon illumination of dried and powdered leaves can be converted into sustained oxygen evolution if mashed leaf material is suspended in a medium containing ferric salts. Analytical determinations indicated that the reaction is:



The early conjecture that this "Hill reaction" represents "photosynthesis with a substitute oxidant" has been since strengthened almost to the point of certainty. Two experiments are particularly convincing: Holt & French (77) showed the isotopic composition of oxygen liberated by the Hill reaction from O^{18} -enriched water to follow that of water—thus proving that this reaction is a photochemical oxidation of water; and Clendenning & Ehrmantraut (78) and Ehrmantraut & Rabinowitch (65) found that the kinetic characteristics of the Hill reaction are very similar to those of photosynthesis. These common characteristics include the maximum rate in steady light per unit chlorophyll amount, the maximum yield obtainable from a (practically) instantaneous light flash, the length of the dark interval required to obtain this maximum flash yield, and the maximum quantum

yield in steady light. The inference is that the two reactions have in common both the primary photochemical process and the enzymatic reaction that limits the over-all rate in strong light.

Preparations of chloroplasts.—Chloroplast preparations used in the study of the Hill reaction are rather unstable and vary strongly in activity. Crude leaf macerates, freed only from coarse particles, often are quite active but lose their activity more or less rapidly, probably depending on the composition of the cell sap. Purer preparations, obtained by precipitating from this mash (by rapid centrifugation) whole and broken chloroplasts and re-suspending them in a buffer, sometimes are less efficient than crude macerates (measuring efficiency by the initial rate of oxygen production in strong light per unit chlorophyll content), but keep their activity longer. Mechanical dispersion of this chloroplast material leads to fractions containing more or less uniform particles. These had been sometimes described as "grana suspensions" [cf. Warburg & Lüttgens (79); Aronoff (80)]; however, there is no proof that they consist entirely of the grana, as described in the section on the structure of the photosynthetic apparatus, and carry no material from the stroma. The specific efficiency has a general tendency to decrease with progressive breaking of the chloroplast material into smaller and smaller fragments [Holt, Smith & French (81); French *et al.* (82)]. A partial restoration of activity lost by dispersion was noted by French *et al.* (82) when extremely fine particles were coagulated to somewhat larger ones by the addition of salts.

Kumm & French (83) and Clendenning & Gorham (84) found only a few plant species (among them millet, flax, Swiss chard, spinach, and lettuce) giving relatively stable and active chloroplast preparations. Broad leaf tree leaves and conifer needles proved unsuitable; material from disintegrated *Chlorella* cells lost its activity within a few minutes.

Various methods of disintegrating cells have been studied, including ultrasonic waves [French *et al.* (85)], squeezing through a needle valve [French *et al.* (82)] and sudden release of high pressure. Arnold & Oppenheimer (86) disintegrated blue-green algae by squeezing them between cylinder and barrel of a syringe.

To preserve activity, chloroplasts must be kept cool during disintegration. [Ch_{10} of deactivation is as high as 6.4, according to French, Anson & Holt (87)]. Activity is lost instantaneously at $+55^{\circ}\text{C}$. [Arnon & Whatley (88)]; the loss remains rapid even at 0°C . Stirring, shaking, or dilution accelerate deactivation. Gorham & Clendenning (89) found that activity, can be preserved indefinitely by snap-freezing in 0.5 *M* sucrose solution, storing at -40°C ., and thawing rapidly at room temperature. Holt, Smith & French (90) noted moderate stabilization by 10 per cent propylene glycol; French *et al.* (82) claimed better results with 10 to 15 per cent methanol. Warburg & Lüttgens (79), noting that potassium chloride restored the activity of chloroplast preparations lost by dialysis, called it the "co-enzyme" of the "Hill enzyme," but Arnon & Whatley (88) found that chloride (which is not

a necessary micronutrient of plants) is not actually required for the Hill reaction. However it appears to protect the separated chloroplast material from inactivation in light.

Hill reaction with different oxidants.—Hill (76), who found ferric oxalate the most convenient oxidant, recommended addition of ferricyanide to reoxidize the ferrous oxalate formed (thus preventing its back reaction with oxygen). Holt & French (91) found that ferricyanide itself can serve as oxidant, without intermediary of ferrus oxalate. Warburg & Lüttgens (79) discovered that *o*-benzoquinone and α -naphthaquinone sulfonate also are reduced by chloroplasts in light; Aronoff (80) added to the list several other naphtha- and anthraquinones; Gurevich (92), *o*-dinitrobenzene; and Holt & French (93), chromate (although the results with the latter oxidant were not clear-cut.) Good results were obtained by Holt & French with several dyes of comparatively strong oxidizing power, such as phenol indophenol, $E_0 = +0.254$ v.; 2, 6-dichlorophenol indophenol, $E_0 = +0.247$ v.; and *o*-cresol indophenol, $E_0 = +0.127$ v. (all potentials for pH 6.6). With thionine ($E_0 = +0.074$ v. at pH 6.6), the results were doubtful; with 1-naphtol-2-sulfo-indophenol ($E_0 = +0.147$ v.), methylene blue ($E_0 = +0.024$ v.), and several indigo sulfonates ($E_0 \approx 0.0$ v.), negative. Holt, Smith & French (81) listed seven additional dyes (of unspecified potentials) as effective "Hill oxidants."

Mehler (94) produced indirect but rather convincing evidence that oxygen can act as Hill oxidant, being itself reduced to H_2O_2 . The proof was in two parts. First, he showed that addition of ethanol and catalase to an anaerobic Hill reaction system (with quinone as oxidant) reveals no formation of H_2O_2 as oxygen precursor (H_2O_2 , C_2H_5OH and catalase are known to produce acetaldehyde, easily detectable by sensitive reactions). He then found that aldehyde is produced, under the same conditions, if quinone is left out but oxygen is present. The logical inference is that H_2O_2 is formed, in this case, as an intermediate in the reduction of O_2 to H_2O (and not of the oxidation of H_2O to O_2). This supports the suggestion (20, p. 543) that photoautooxidations *in vivo* occur by oxygen taking the place of carbon dioxide as hydrogen acceptor in photosynthesis.

It was natural to look for effective Hill oxidants among biological substances known to serve as intermediates in the "downgrade" movement of hydrogen in respiration. One could hope to find in this way a gate through which hydrogen (which light had taken away from water and lifted to a reasonably high level of reducing potential) could be forced back into the familiar paths of respiratory intermediates, and perhaps made to back up through these paths all the way up to carbohydrates—simply by dint of crowding the gate densely enough.

The successfully employed nonbiological Hill oxidants all had positive potentials. (In fact, most of them were above $+0.2$ v.) The best-known biological redox intermediates are (DPN and TPN which have potentials of approximately -0.35 v., and the cytochromes, which have potentials from $+0.26$ v. downwards. Holt (95) was able to demonstrate, by use of spectral

analysis, that cytochrome-*c* is reduced by chloroplasts in light, but he could find no equivalent liberation of oxygen, apparently because cytochrome-*c* oxidase is present in such amounts as to cause rapid back reactions between oxygen and reduced cytochrome-*c*. A photostationary state is thus established in which the amount of reduced cytochrome-*c* is sufficient for spectroscopic detection, but the amount of oxygen is too small for manometric measurement. According to Mehler (94), Tolmach in Chicago was able to detect liberation of oxygen in the cytochrome + chloroplasts system by poisoning the cytochrome oxidase with sodium azide.

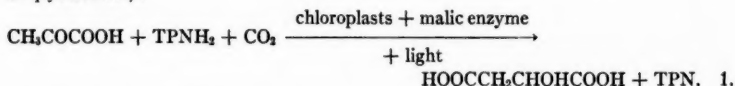
The potential of the oxidant in photosynthesis is about -0.5 v. (free CO_2) or -0.4 v. (CO_2 incorporated into a carboxyl group), while that of the reductant is as high as $+0.8$ v. (The difference of 1.3 v. corresponds to the large positive free energy of photosynthesis, $-\Delta F^\circ = 1.3 \times 23 \times 4 = 119$ kcal. per mole CO_2 , where 4 is the number of electrons or hydrogen atoms to be transferred, and 23 the conversion factor from electron volts to kcal. per mole.) Therefore, the reduction of an oxidant with a potential of 0.2 v. is equivalent to light-induced transfer of hydrogen from water to an acceptor about halfway up to carbon dioxide. With the reduction of a pyridine nucleotide ($E_0 = -0.35$ v.), on the other hand, almost 90 per cent of the potential gap would be bridged. In first experiments with pyridine nucleotides as Hill oxidants, no reduction was noted spectroscopically, and no oxygen evolution was observed manometrically [Mehler (94); French & Holt (96)]. However, Ochoa & Vishniac (97), and Tolmach (98), working in Franck & Gaffron's laboratory, have found that if the pyruvate, carbonate, TPN, and so-called "malic enzyme" (which was discovered by Ochoa in pigeon liver, but also present in many other animal and plant tissues) that catalyzes oxidative decarboxylation of malate to pyruvate are brought together with a chloroplast suspension and illuminated, the formation of malate can be discovered by enzymatic tests (by decarboxylation with the same "malic enzyme"), and the formation of oxygen can be demonstrated by reduction of chromous chloride [Ochoa & Vishniac, (97)] or by phosphorescence quenching [Tolmach (98)]. The fixation of carbon in the chloroplast material also can be detected under these conditions by C^{14} -tracer [Ochoa & Vishniac (97); Tolmach (98)].

Annon (38) has found that if the malic enzyme derived from the cytoplasmic material of mashed and centrifuged sugar beet leaves is added to chloroplast preparations from the same leaves (together with Mn-salt, TPN, pyruvate and bicarbonate), oxygen liberation and malic acid formation can be observed in light (by manometry and chromatographic identification with C^{14} , respectively).

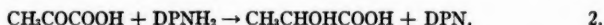
This lends some additional plausibility to the hypothesis that photosynthesis takes the path through the malic enzyme system. However experiments with tracers (not discussed in this review) have demonstrated that early formation of tagged malic acid, while a common occurrence, is not a rule; moreover, tagging of malic acid can be inhibited without affecting the formation of tagged carbohydrates.

In the chloroplast-TPN-pyruvate-malic enzyme system, TPN must serve as the primary hydrogen acceptor; rapid reoxidation of TPNH_2 , either by free oxygen or by its postulated precursors in photosynthesis—sometimes named “photoperoxides”—, must be the reason why no Hill reaction could be detected in systems containing only chloroplast material and TPN. In the TPN-“malic enzyme” system, hydrogen seems to be transferred from reduced TPN (TPNH_2) to pyruvate so rapidly that this transfer competes effectively with the back reaction of TPNH_2 with the “photoperoxides.” (Free oxygen is eliminated, in Tolmach’s experiments, by a fast stream of pure nitrogen, and in Vishniac’s experiments, by absorption in chromous chloride.)

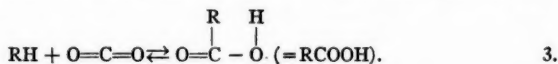
The ultimate oxidant in the malic enzyme system is the carbonyl group in pyruvic acid (and not the carboxyl group, formed by addition of CO_2 to pyruvate!):



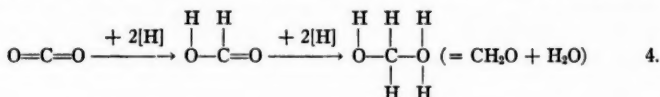
Ochoa & Vishniac (97) also observed the formation of lactic acid in light from pyruvic acid, chloroplast material, and DPN—a reaction that is similar to that produced in the malic enzyme system, except that the reduction of the carbonyl group occurs without coupled carboxylation:



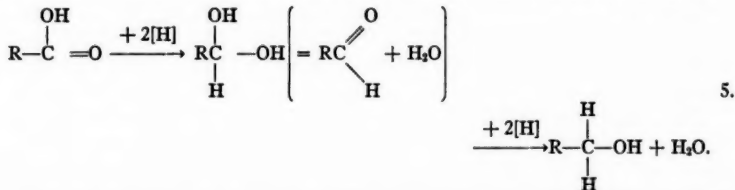
There is little doubt that the first reaction of carbon dioxide in photosynthesis is the addition of an organic molecule, RH , to a $\text{C}=\text{O}$ bond in CO_2 :



The first two steps in the reduction of carbon dioxide to carbohydrate

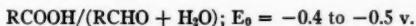


are thus replaced by the first two steps in the reduction of a carboxyl:



Since carboxylation has a comparatively small free energy (5 to 10 kcal. per mole), the two steps of reaction 5 require only slightly less energy

than those of reaction 4. In both reaction sequences, the first step is more difficult than the second one (carbon-oxygen bonds are generally strengthened by accumulation at a single carbon atom). Approximately, the potentials of the two steps in reaction 5 are:



Reduced TPN or DPN ($E_0 \approx -0.35 \text{ v.}$) has enough reducing power to bring about the second, but not the first step.

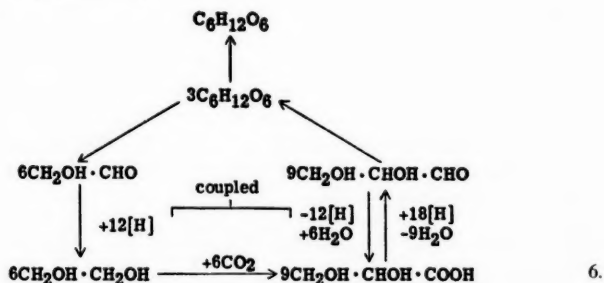
It has been suggested that insufficient reduction potential of an intermediary reductant can be remedied by the maintenance of an extremely high relative concentration of its reduced form (or of an extremely low relative concentration of the reduced form of the final hydrogen acceptor). In our case, that would mean that the reduction of RCOOH to RCHO + H₂O could be achieved by TPNH₂, if photochemical reduction of TPN would keep the concentration ratio ([TPNH₂]/[TPN]) very high; while the removal of the reduction product, RCHO, by an appropriate chemical or physical agent, would keep the ratio [RCHO]/[RCOOH] very low. However, the possibilities of such "over-the-hump" reaction paths should not be exaggerated.

Back reactions of TPNH₂ with the photochemical oxidation products of water must hamper the maintenance of a high ratio [TPNH₂]/[TPN]. It was assumed above that TPNH₂ reacts with the malic enzyme system fast enough to compete with these back reactions; but if the reduction of RCOOH by TPNH₂ is strongly endothermic—as it should be if the ΔF of the reaction is strongly positive—it cannot be fast. Furthermore, if the reaction is to proceed with a good yield, enough TPN molecules must be present in the photostationary state to give all excited chlorophyll molecules a chance to react with them; this, too, precludes the maintenance, during photosynthesis, of an extremely low ratio [TPN]/[TPNH₂]. For these two reasons, it seems unlikely that the reduction of carboxyl to carbonyl can be mediated, in photosynthesis, by a catalyst, such as TPN, whose normal potential is 0.1 to 0.2 v. more positive than that of the carboxyl-carbonyl system.

There are two possibilities to get around this difficulty: either the use, instead of pyridine nucleotide, of another intermediate hydrogen acceptor with a stronger reducing power (i.e., a more negative potential), or the coupling of this oxidation reduction with an energy-supplying process (such as the degradation of a high energy phosphate). The first alternative means making better use of light energy; the second, supplementing light energy with chemical energy derived from an exergonic process, such as normal respiration or reoxidation of some intermediate product of photosynthesis. Since respiration substrates are themselves products of photosynthesis, this alternative means the supplementing of the energy of one light quantum by that of another quantum, absorbed earlier. This possibility was discussed in (20, chapter 9) under the name of "energy dismutation."

Kok (99) and van der Veen (100) have suggested mechanisms of photo-

synthesis in which "energy dismutation" was achieved by partial reoxidation of the products of photosynthesis, and conversion of the liberated oxidation energy into phosphate bond energy. Gaffron, Fager & Rosenberg (101) proposed another mechanism based on the energy dismutation principle; in this scheme, a C_2 -product of high degree of reduction, was supposed to be generated in photosynthesis from a part of the photosynthesized hexose, the energy needed for reduction being provided by a coupling of this endergonic process with the reoxidation of a part of the photosynthesized triose back to pyruvic acid (equation 6).



Warburg and co-workers (102) also stipulated an energy dismutation mechanism in interpreting the results of their quantum yield determination in light of alternating intensity. They concluded from these measurements that the energy of a single light quantum is supplemented, in the reduction of a molecule CO_2 , by the energy of (strongly enhanced) respiration. Warburg found evidence of this enhancement in the measurement of oxygen consumption in the first minute after the cessation (or reduction) of illumination. (During illumination, enhanced respiration is difficult if not impossible to distinguish from reduced photosynthesis.) Whether one accepts Warburg's interpretation of the experimental results or not, the characterization of these results as proving that photosynthesis is a "one quantum" process is misleading, since it makes a casual reader believe that the quantum requirement of the reaction $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \{\text{CH}_2\text{O} + \text{O}_2\}$ has been reduced to 1, which would contradict the law of conservation of energy. No such claim was made by Warburg or Burk; their claim is that photosynthesis can occur with the minimum quantum requirement permissible under the energy conservation law (which means about 2.6 quanta per CO_2 molecule) but that only one of these 2.6 quanta has to be supplied at the exact time of the measurement, while the energy of the other 1.6 quanta can be derived from accelerated reoxidation of photosynthates during 1 min. (or a few min.) preceding the measuring period.²

Returning to the Hill reaction, we note that it has now been successfully

² We do not discuss the quantum requirement problem in this review. With the exception of the 1951 papers by Warburg *et al.* (102), the pertinent papers have been reviewed in (103).

coupled with a reaction which could (but does not necessarily) represent the combination of the first carboxylation step with the less difficult of the two reduction steps in photosynthesis: $\text{RCHO} \rightarrow \text{RCH}_2\text{OH}$. Preliminary phosphorylation of the carboxyl group by a high energy phosphate should make it possible to link the Hill reaction, *in vitro*, also with a reaction (e.g., the reduction of glyceric acid to glyceraldehyde) analogous to (but again not necessarily identical with) the more difficult reduction step of photosynthesis ($\text{RCOOH} \rightarrow \text{RCHO}$).

It has been thus made plausible that photosynthesis could proceed via the reduction of TPN (or DPN), coupled with the formation of high energy phosphate, such as adenosinetriphosphate (ATP), by partial reoxidation of the photochemical reduction products; but there is, as yet, no direct evidence that photosynthesis actually involves as intermediate TPNH_2 or DPNH_2 , or that high energy phosphate plays a role in it.

Boichenko (104) claimed that deposits of leaf mash on filter paper, suspended in carbonate solution, produce oxygen in light, if the medium is made reducing by the addition of fructose. The observed decrease of pH in light was taken as indication of the reduction of carbon dioxide. Later, Boichenko (105) asserted that the same preparations can reduce carbon dioxide (to formic acid) also in the dark, if molecular hydrogen is provided as reductant. Boichenko's experimental techniques were crude and she used very implausible concepts in the interpretations of her observations.

In Hill's first investigation (76) it was noted that the amount of oxygen, produced by chloroplast preparations in light without added oxidant, was decreased by washing the chloroplasts from the cell juice, and enhanced again by the addition of this juice. Clearly, the cell juice contained an unknown oxidant, X—conceivably the same as the hypothetical primary hydrogen acceptor in photosynthesis. It would be of obvious interest to identify X—but so far, not much progress has been made toward this aim.

Franck (106) noted that the presence of carbon dioxide prolonged evolution of oxygen by leaf preparations without added oxidants; but using labeled CO_2 , Brown & Franck (107) could find no C^{14} -fixation by chloroplasts under these conditions, thus disposing of the tempting hypothesis that reduced X is capable of transferring hydrogen to carbon dioxide even after the cell structure has been smashed.

Hill, Davenport & Whatley (108) noted that crude, unwashed chloroplast preparations reduce methemoglobin in light with the production of oxygen, while methemoglobin does not act as Hill oxidant with washed chloroplasts. Addition to washed chloroplasts of aqueous extract from acetone-treated leaves restored the capacity to reduce methemoglobin. The factor (compound X?) apparently needed to mediate between the Hill reaction system and the methemoglobin-hemoglobin system, was found to have the characteristics of a protein [it was thermolabile, not dialysable, stable between pH 5 and 9, and was precipitated only by nearly-saturated $(\text{NH}_4)_2\text{SO}_4$].

Kinetics of the Hill reaction.—Various methods for measuring the Hill reaction have been proposed (in addition to manometry), among them

photometry [with dyes as oxidants, see Holt, Smith & French (90)]; pH measurement [e.g., with ferric compounds as oxidants, see Clendenning & Gorham (84); and Holt & French (91)]; and redox potentiometry [see Spikes *et al.* (109)].

The quantum requirement of the Hill reaction was first measured by French & Rabideau [in (106)], using ferric oxalate as oxidant. Results scattered from 15 to 50 quanta per molecule of oxygen. More consistent results were obtained by Ehrmantraut & Rabinowitch (65) with quinone. Using red light (to avoid absorption of light by quinone), quantum requirements of 9.3 to 11.5 were measured with chloroplasts from *Phytolacca americana*, by comparison of the oxygen production in light with the oxygen consumption in a Warburg-Schocken actinometer. With Hill's solution (ferric oxalate + ferricyanide), the quantum requirement of chloroplasts was 12.7 quanta per molecule O_2 ; with ferricyanide alone, 11.0. (See below regarding the quantum requirement of the quinone reduction by whole *Chlorella* cells.)

The maximum rate of the Hill reaction in saturating light can reach [after Arnon & Whatley (88); Clendenning & Gorham (84); Kumm & French (83); and Holt, Arnold & Brooks (110)] one mole oxygen every 30 to 40 sec. per mole chlorophyll. According to Willstätter & Stoll, the "assimilation time" of green plants is somewhat shorter—one molecule oxygen from one molecule chlorophyll in 15 to 20 sec. In a more direct comparison, Clendenning & Ehrmantraut (78) found that *Chlorella* cells produce, at saturation, practically equal volumes of oxygen in carbonate buffer and in phosphate-buffered quinone solution (cf. section on Hill reaction in *Chlorella* cells below).

Hill & Scarisbrick (76) and Aronoff (80) noted that the Hill reaction is not inhibited by cyanide; Ehrmantraut & Rabinowitch (65) found the same for quinone reduction by *Chlorella* cells. This supports the hypothesis—cf., for example, Weller & Franck (111)—that, in photosynthesis, cyanide is a specific poison for the carboxylation enzyme, not involved in the Hill reaction.

Inhibition of the Hill reaction by azide and hydroxylamine was noted by French, *et al.* (85), Arnon & Whatley (88) and Macdowall (112), but not by Hill & Scarisbrick (76) and Aronoff (80). Clendenning & Gorham (84) reported the hydroxylamine inhibits the Hill reaction with ferric salts, but not with quinone; but Arnoñ & Whatley (88) found inhibition with quinone, too. If—as conjectured by Gaffron (113) and Weller & Franck (111)—hydroxylamine is a specific poison for the oxygen-liberating enzyme, it should inhibit also the Hill reaction.

The Hill reaction is highly sensitive to *o*-phenanthroline [see Warburg & Lüttgens (45); Aronoff (80); Arnon & Whatley (88); Macdowall (112)] and to dinitrophenol [Macdowall (112)]; but insensitive to carbon monoxide [Warburg & Lüttgens (45)]. It is inhibited by narcotics (such as phenylurethan), and by Cu^{++} and Hg^{++} ions. Holt, Brooks & Arnold (110) found practically identical inhibition by ultraviolet light (253.7 $m\mu$) of photosyn-

thesis and of the Hill reaction, in *Chlorella* cells and in chloroplasts, and in weak and in strong light. Equal inhibition at all light intensities appears, at first sight, not particularly remarkable; but if photosynthesis (and the Hill reaction) are photochemical processes combined with kinetically independent enzymatic reactions, the identity of inhibitory effects—whether of ultra-violet light, or of poisons such as hydroxylamine, or of physiological factors such as age—in weak light (where the rate is limited by the number of photochemically active chlorophyll molecules), and in strong light (where it is limited by the number of catalytically active enzyme molecules), requires special interpretation. It seems to indicate preferential co-operation of a given molecule of the rate-limiting enzyme with a given molecule (or group of molecules) of chlorophyll; according to flashing light experiments of Emerson & Arnold (114), this group may contain about 10^3 chlorophyll molecules. Whenever a molecule of the limiting enzyme is “knocked out,” the chlorophyll molecules that normally supply it with substrate become useless.

Davis (115) showed that *Chlorella* mutants can be produced by ultra-violet irradiation which are capable of the Hill reaction but not of photosynthesis or are incapable of both functions, despite a normal content of apparently unchanged chlorophyll.

Hill reaction in Chlorella cells.—That “Hill reaction” (meaning now by this term water oxidation by oxidants other than carbon dioxide) can occur also in whole *Chlorella* cells was first asserted by Fan, Stauffer & Umbreit (116). Somewhat unexpectedly, benzaldehyde seemed to give the best results. However, Ehrmantraut & Rabinowitch (65) could find no oxygen liberation by *Chlorella* in the presence of benzaldehyde. On the other hand, the observation of Warburg & Lüttgens (45) that *Chlorella* cells liberate oxygen in light, in the presence of *o*-benzoquinone, has been fully confirmed. Holt & French (93) noted oxygen liberation also with ferricyanide and ferric oxalate, but Ehrmantraut & Rabinowitch (65) found this to be a result of the photo-oxidation of oxalate to carbon dioxide, followed by normal photosynthesis; as expected, oxygen evolution is, in this case, inhibited by cyanide. Thus, at present, the only known effective oxidant for *Chlorella* cells is quinone—an unsatisfactory situation because of the poisonous effect of this compound, which inhibits photosynthesis irreversibly, and also affects respiration.

For the quantum requirement of oxygen production by *Chlorella* in phosphate buffer with quinone as oxidant, Ehrmantraut & Rabinowitch (65) found values of 11 to 13 by comparison with the Warburg-Schocken actinometer, and 10.4 ± 1.5 by the more reliable bolometric energy measurement in monochromatic bands. The maximum rate of oxygen production in strong light is, according to Clendenning & Ehrmantraut (78), very close to that of the same cells in carbonate buffer; however, much stronger light is needed to reach this saturation plateau with quinone than with carbonate.

The effect of quinone on chlorophyll fluorescence *in vivo*, described by Shiau & Franck (117), indicates that quinone becomes attached to chloro-

phyll. The strong quenching of chlorophyll fluorescence *in vitro* was noted by Livingston & Ke (118) and was attributed to the oxidative properties of quinone (since all strong quenchers proved to be oxidants).

Clendenning & Ehrmantraut (78) noted the absence, in the Hill reaction of *Chlorella* with quinone, of the induction losses observed in the first minutes of photosynthesis. According to the Franck-Gaffron theory of induction, this means that quinone, attached to chlorophyll, is not displaced from the latter by "narcotics," which Franck assumes are formed in the first seconds of exposure to light, blanket chlorophyll and thus keep it photochemically inactive until the oxygen-liberating enzyme, which has become deactivated in the dark, is reactivated and normal photosynthesis can get under way.

PHOTOCHEMISTRY OF CHLOROPHYLL SOLUTIONS

Chloroplasmic material in colloidal suspension can be considered as intermediate between chloroplasts in the living cell and chlorophyll preparations *in vitro*. This material resembles the cell in its capacity to sensitize oxidation-reduction reactions in which light energy is converted into potential chemical energy, and to use water as hydrogen donor in such reactions. The next question is whether a similar activity can be initiated also by using chlorophyll in true solution or in colloidal form. A preliminary question here is whether chlorophyll *in vitro* is capable of reversible oxidation (or reversible reduction, or both), since in the usual mechanism of sensitization of oxidation-reduction reactions, the light-excited sensitizer first reacts with one reactant (which can be the oxidant, or the reductant) and is then restored to its original state by reaction with the other reactant. In this way, the sensitizing pigment serves as a light-activated intermediate oxidation-reduction catalyst.

An alternative mechanism is, however, possible if the sensitizer is combined with both the oxidant and the reductant in a more or less stable complex. In this case, excitation energy can be used to transfer an electron (or, in the final result, a hydrogen atom) from the reductant to the oxidant in a single step—even though this transfer may proceed via the molecule of the sensitizer. The concept of "physical" sensitization, in which the photocatalyst serves only as energy supplier, merges here with the concept of "chemical" oxidation-reduction catalysis, in which the catalyst undergoes a reversible oxidation-reduction.

Reversible reduction of chlorophyll?—Many dyestuffs are reducible to colorless "leuco dyes" and can use light energy to increase their oxidizing capacity. (Oxidation of ferrous ions by the dyestuff thionine in light, which is reversed in the dark, is a good example.) Chlorophyll does not form a leuco compound with the same ease. Timiriazev (120) did observe in 1903 that chlorophyll is reduced by zinc and acetic acid to a brownish compound that slowly becomes green again in air; Kuhn & Winterstein (121) found that the products of this cycle are identical with the original pigments for both chlorophyll *a* and chlorophyll *b*; but Rothmund and co-workers (122) noted that the final product differs from the original pigment in its fluorescence

spectrum and its degradation products; reduction and reoxidation of chlorophyll thus leave an irreversible residual change. It has been suggested that this change consists in the hydrogenation of the vinyl side chain, and perhaps also a splitting of the cyclopentanone ring, converting a "phorbin" into a "meso-chlorin." A different conclusion was reached by Kosobutskaja & Krasnovsky (123) in a recent repetition of the Timiriazev reaction, with chlorophyll [(a+b), a, b], pheophytin (a+b), Zn-chlorophyllide [(a+c), a, b], Cu-chlorophyllide (a+b), and Mg-phthalocyanin (all in pyridine solution). In all cases, reduction led to compounds with almost no red absorption band and a weakened blue-violet band; only a very slight indication of an absorption band at 525 $m\mu$, typical of photo-reduced chlorophyll *a*, could be noticed. Reoxidation in air lead to products whose spectroscopic and chromatographic study indicated identity with Zn-chlorophyllide (absorption peaks at 661 and 431 $m\mu$ in pyridine). With Zn-chlorophyllide as initial material, regeneration was practically complete; with Cu-chlorophyllide, the final product probably was a mixture of the Zn and Cu derivatives. About one-half of the initial chlorophyll amount was not reoxidized at all, but irreversibly decomposed into greyish products.

More significant than the reduction by zinc and acid (from the point of view of the function of chlorophyll in photosynthesis) is its reversible photochemical reduction. This has been investigated in a series of papers by Krasnovsky and co-workers.

Krasnovsky (124) noted that chlorophyll solutions in pyridine are reversibly decolorized if illuminated in the presence of ascorbic acid under anaerobic conditions. No bleaching occurs if alcohol or acetone are used as solvents. In pyridine, the fluorescence and the green color disappear after a few minutes illumination with a 500 w. lamp and gradually return in darkness; the return is accelerated by admission of air. The bleached solution is pink and has a characteristic absorption band at 525 $m\mu$. If the reaction is carried out with chlorophyll *a+b*, the absorption spectrum after the completion of the back reaction is somewhat changed—it seems to lack the blue band of chlorophyll *b*. With pure chlorophyll *a*, the final spectrum is very similar to that before the reaction (except for evidence of a slight formation of pheophytin).

Krasnovsky suggested that the pink product may be a semiquinone rather than a fully reduced leuco compound. The importance of a basic solvent for its appearance could then be attributed to the formation of a comparatively stable ionic form of the semiquinone in a medium of high proton affinity. In a second paper, Krasnovsky (125) found that the regeneration of chlorophyll after photochemical reduction by ascorbic acid ($E_0 = +0.05$ v. at pH 7.0) is accelerated by the addition of riboflavin ($E_0 = -0.22$ v.) or safranin T ($E_0 = -0.29$ v.). The end result of these reactions must be chlorophyll-sensitized reduction of these compounds by ascorbic acid, a reaction which does not occur in the dark because it involves a considerable increase in free energy. Chlorophyll serves here as light-activated intermediate catalyst. The whole reaction is slowly reversed in the dark; admission of

oxygen accelerates the regeneration of the oxidant (safranin or riboflavin). Krasnovsky & Brin (126) made similar experiments with DPN as the ultimate hydrogen acceptor. By subtraction of the original absorption curves in the near ultraviolet from those after the illumination (of a mixture of chlorophyll, ascorbic acid, and 60 per cent pure DPN, in pyridine), they obtained evidence that a new absorption band, with a peak at $340\text{ m}\mu$ was formed in light. This is the location of a characteristic band of DPNH_2 in pyridine.

The important conclusion that a compound with an oxidation potential as low as that of DPN (about -0.35 v.) can be reduced in light at the expense of ascorbic acid ($E_0 = +0.05\text{ v.}$) was further supported by demonstrating that reoxidation of the pink product of photochemical chlorophyll reduction is accelerated by the addition of DPN (as it is by oxygen, riboflavin, or safranin T). However, the red peak of chlorophyll absorption could be restored by DPN only to about one-third of its original intensity, as against two-thirds in the case of riboflavin.

Krasnovsky & Brin suggested that solvation of chlorophyll by pyridine is analogous to the attachment of a hemochromogen to a protein which occurs by a link between the Fe-atom and an N-atom in an imidazole ring, a type of complexing that enhances the capacity of the iron porphyrin for reversible oxidation-reduction.

Krasnovsky, Brin & Vojnovskaja (127) investigated more systematically the influence of solvent on the photoreduction of chlorophyll *a* and *b* by ascorbic acid. They found that in pyridine, the red absorption peak was reduced by 90 per cent, and that 80 per cent of this bleaching was reversed in the dark. Dilution of pyridine with water decreased both the extent and the reversibility of bleaching. In aniline, the red peak was reduced in light only by 22 per cent, and only half of this bleaching was reversed in the dark. In ethanol, the corresponding figures were 20 per cent and 14 per cent; in acetone, 29 per cent and 19 per cent. Addition of as little as 0.3 per cent pyridine, imidazole, or histidine, to alcohol or acetone, increased the bleaching to 80 per cent, with one-half of it being reversed in the dark. Twenty-six organic and two inorganic compounds were tried out as reducing agents in pyridine. Positive results were obtained only with ascorbate, dioxymaleate, cysteine, phenylhydrazine and H_2S ; pyruvic acid (earlier mentioned by Krasnovsky as giving results similar to those produced by ascorbic acid) was now listed among compounds giving negative results.

Renewed investigation of chlorophyll *b* showed that its reduction by ascorbic acid in light and reoxidation in dark gives a product with absorption bands at $693\text{ m}\mu$ and $432\text{ m}\mu$, which is neither chlorophyll *a* nor pheophytin *a* or *b*. (Conversion of chlorophyll *b* to chlorophyll *a* in this reaction was suggested in an earlier paper as a possibility.)

Krasnovsky & Brin (128) made a similar review of different oxidants for the reoxidation of photochemically reduced chlorophyll. The following compounds with positive oxidation potentials were found to accelerate the return of absorption in the red peak (in order of decreasing efficiency): thionine

(5×10^{-4} M), quinone (1×10^{-2} M), methylene blue (5×10^{-4} M), phenol indophenol (5×10^{-4} M), and dehydroascorbic acid (5×10^{-2} M). The last result shows that the other oxidants—although they do react with ascorbic acid—do not act on reduced chlorophyll via preliminary dark reaction with ascorbic acid. Among compounds not reacting with ascorbic acid in the dark, the order of effectiveness in the re-oxidation of reduced chlorophyll was: hematin (5×10^{-4} M), $\text{NO}_2^-(10^{-2}$ M), $\text{NO}_3^-(10^{-2}$ M), $\text{Fe}^{+3}(10^{-2}$ M), $\text{Cu}^{++}(10^{-2}$ M), and air. Among compounds with negative potentials, safranin T, Neutral Red, Nile Blue (all 5×10^{-4} M) gave positive results; also 5×10^{-4} M riboflavin, and 1×10^{-3} or 5×10^{-4} M DPN ($E_0 = -0.32$ v.). Since xanthin ($E_0 = -0.37$ v.) showed no accelerating influence, the authors concluded that E_0 of the system chlorophyll-reduced chlorophyll must be about -0.35 v. (It should be noted, however, that the quoted potentials had been measured in water, while the observations of Krasnovsky & Brin were made in pyridine!)

Of seven fatty acids tested, only 10^{-2} M malic acid produced marked acceleration. Temperature had little effect on the rate of the back reaction—a result that was taken as confirmation of the hypothesis that the pink reduced form of chlorophyll is a semiquinone and, as such, is able to react with a very small energy of activation. (If this is the case, however, the low absolute rate of reaction becomes puzzling.)

Evstigneev & Gavrilova (129) noted that chlorophyll and Mg-phthalocyanine are reduced by ascorbic acid in light in pyridine solution slower than the corresponding Mg-free compounds (but are photoautoxidized faster than the latter in methanolic solution!)

As long as Krasnovsky's assumption of a reversible oxidation-reduction of chlorophyll rests only on the observation of a sometime far-reaching, but never complete, restoration of the extinction coefficient in the peak of the red band, some doubt remains whether this assumption is correct and the spectral change at the end of the cycle is merely the result of a side reaction, or whether the main reaction itself involves an irreversible transformation of chlorophyll (in which case, it could not serve as basis for catalytic activity). It is of some interest, therefore, that Holt (130), in checking Krasnovsky's experiments with ascorbic acid and quinone, was able to repeat the bleaching and recoloration cycle three or four times with the same sample. True, the restored red band became weaker with each cycle but, nevertheless, the result is hardly compatible with the assumption that after a cycle, all chlorophyll molecules that took part in it are left with a permanent change in their structure or composition. It seems more likely that the reaction is basically reversible, but that a certain proportion of chlorophyll molecules that take part in it, undergo irreversible side reactions, which lead to the loss of the red absorption band. It would be important to find a way to suppress these irreversible reactions *in vitro* as effectively as they appear to be suppressed *in vivo*.

A more precise kinetic study of an oxidation-reduction reaction sensitized by chlorophyll in solution was made by Livingston and co-workers. This

reaction—the reduction of methyl red by phenylhydrazine—was first investigated by Ghosh & Sen-Gupta (131). Livingston, Sickle & Uchiyama (132) first found for it a quantum requirement of 6 to 10 (quanta per molecule reduced methyl red), independently of methyl red concentration. In a more detail study, Livingston & Pariser (133) obtained quantum requirements down to 2 quanta per molecule, and found them to be a function of the concentration of phenylhydrazine (with saturation at about 10^{-1} mole/l.), as well as of the concentration of the neutral form of methyl red with saturation at about 2×10^{-4} mole/l. (The three forms of this dye are: anion, neutral molecule or zwitterion and cation.) In the first paper, Livingston had suggested a reaction mechanism involving reversible reduction of chlorophyll by phenylhydrazine (after preliminary conversion into a long-lived metastable state); in the second paper, he gave a more quantitative kinetic interpretation on the basis of a different mechanism, bearing more similarity to reversible oxidation than to reversible reduction, in that it assumed that light-activated chlorophyll first reacts with the oxidant (methyl red). However, Livingston postulated that this reaction is not an oxidation-reduction, but merely a complex formation between metastable chlorophyll and methyl red; the two travel together until they meet a molecule of phenylhydrazine. The encounter leads to hydrogen transfer from the latter directly to methyl red, with the help of the excitation energy of chlorophyll. (This is a "physical" rather than a "chemical" sensitization mechanism, as defined above). However, it seems that the kinetics of this process would be about the same if one would assume that metastable chlorophyll is oxidized by methyl red in the first encounter and reduced by phenylhydrazine in the second one. Since the reaction is carried out in methanol, it seems plausible that its mechanism should be similar to that of other reactions in this medium that are to be discussed in the following section and tentatively interpreted there as involving a reversible oxidation of chlorophyll.

Reversible oxidation of chlorophyll?—While chlorophyll in a basic solvent, such as pyridine, exhibits a capacity for reversible reduction, the same compound shows in other solvents, such as methanol or ethanol, a pronounced tendency for reversible oxidation. This type of reaction was first postulated by Rabinowitch & Weiss (134) in the interpretation of the reversible change of color of methanolic chlorophyll (or chlorophyllide) solutions caused by the addition of ferric (or ceric) salts. The color is slowly restored in the dark and returns immediately upon addition of excess ferrous chloride or of hydroquinone. Rabinowitch found the bleaching reaction to be accelerated by light [(20, p. 488)]. If the reductant is added without delay after the addition of the oxidant, the original chlorophyll spectrum is completely restored; otherwise there remains a permanent reduction in the intensity of the red band (and increase of absorption in the green, indicative of pheophytinization). Attribution of this reversible color change to a reversible oxidation-reduction was criticized by Ashkinazi, Glikman & Dain (135), who observed that (as noted already by Rabinowitch & Weiss) similar color changes could be produced also by nonoxidizing salts; they probably indicate formation of

different metal complexes of chlorophyll. However, the much faster rate of decoloration in the case of Fe^{+3} , the exact restoration of the spectrum, and the possibility of repeating the decoloration and recoloration cycle several times, argue for the original interpretation. (The oxidation-reduction equilibrium, apparently reached by chlorophyll in a ferric-ferrous salt mixture, can be shifted back and forth by alternating darkness and light many times.) Ashkinazi & Dain (136) have prepared a chlorophyll-ferrous iron complex by the action of ferrous acetate on pheophytin and have found that (in ethanol) this complex is oxidized by air with a shift of the absorption from 695 to 610 μ ; upon illumination, the band is shifted back to 645 μ , indicating that the oxidation (presumably to a ferric complex?) is reversed by light.

Reversible photochemical oxidation of chlorophyll has also been assumed by Linschitz (137), who illuminated chlorophyll solutions in an organic solvent mixture frozen into a glass-like solid, in the presence of oxidants such as quinone. He observed a complete loss of color in light, and its restoration upon thawing of the solution in darkness. In the decolorized state, the (presumably oxidized) chlorophyll had only the trace of an absorption band in the red, and no new bands were apparent in the middle of the visible spectrum; a similar spectrum was observed by Rabinowitch & Porret in chlorophyll solutions oxidized by ceric salt [cf. figure in (20), Volume I].

Kachan & Dain (138) froze chlorophyll solutions in ethanol or ethanol-ether (1:3) in liquid air and illuminated with a 500 w. lamp, a high pressure Hg arc, or with an Al (or Cd) spark. Visible light had no effect; in methanolic solution, ultraviolet light also caused only slight bleaching in the red absorption band; but in alcohol-ether, ultraviolet illumination caused the red absorption band to disappear in 20 to 30 min. The bleached product could be preserved at low temperatures, but the red band returned upon melting. This reversible change was assumed to be oxidation (loss of an electron) by chlorophyll: $\text{Chl} + h\nu \rightarrow \text{Chl}^+ + e$ (stuck in medium?) with the oxidized state stabilized by the transfer of a proton to ether to form an oxonium ion: $\text{Chl}^+ + (\text{C}_2\text{H}_5)_2\text{O} \rightarrow (\text{C}_2\text{H}_5)_2\text{OH}^+ + \text{"dehydrochlorophyll."}$

A reversible bleaching of the red band, upon illumination of a methanolic solution of chlorophyll, was observed by Porret & Rabinowitch (139) also without added oxidant, provided oxygen was rigidly excluded. The loss of absorption, in light from a 1500 w. lamp, was of the order of 1 per cent only, but it was well reproducible and bleaching could be repeated indefinitely with the same sample. This reaction was further studied by Livingston & McBrady (140), who have found that the color was restored by a second-order reaction—a result that agrees with the proportionality of the steady state bleaching with the square root of light intensity, noted by Porret & Rabinowitch. (If the forward reaction in this reversible change is photochemical reduction of methanol by chlorophyll, the second-order back reaction could be the reduction of the oxidation product of chlorophyll by the reduction product of methanol.)

To sum up, recent experiments indicate (but do not quite prove) that

chlorophyll is capable of both a reversible oxidation and a reversible reduction; that one is favored in methanol, and the other in pyridine; and that both reactions are accelerated by light (and, with certain oxidants or reductants, are possible in light only). This makes chlorophyll a versatile "photocatalyst" for oxidation-reduction reactions, including reactions occurring against the gradient of the electrochemical potential, and resulting in conversion of light into chemical energy.

If reduction of DPN or TPN by photochemically reduced chlorophyll (asserted by Krasnovsky) could be definitely established, it should be possible, in principle, to tie up this reaction with reductive carboxylation of pyruvate, in the same way as this was done with chloroplasts by Ochoa & Vishniac (97), Tolmach (98), and Arnon (38).

A gap to be bridged yet remains, however, between a reaction of chlorophyll in an organic solvent, such as pyridine, and the enzymatic reactions which require an aqueous medium. One of the functions of the chloroplast structure, described in the first section of this review, may be to fill this gap.

No indications exist as yet that chlorophyll in solution can be used to take hydrogen away from water and thus to liberate oxygen in light—the capacity so characteristic of live plant cells and of chloroplast preparations. The oxygen liberation by these biological materials is an enzymatic process. The enzymes involved in the utilization of carbon dioxide as hydrogen acceptor are lost in passing from live cells to chloroplast preparations. The enzymes permitting the use of water as hydrogen donor are still present in the latter, but are lost in the extraction and purification of the pigment. One could then suggest that while the Hill reaction is one enzymatic step removed from the photosynthesis, the Krasnovsky reaction lacks two such steps. A legitimate alternative postulate is, however, that photosynthesis makes use not only of the capacity of chlorophyll for reversible photochemical reduction, but also of its capacity for reversible photochemical oxidation, so that, after the hydrogen atoms are transferred in a reaction of the type observed by Krasnovsky, i.e., from an intermediate donor (such as ascorbic acid) to an intermediate acceptor (such as riboflavin or DPN), their replacement occurs by a second photochemical reaction, in which hydrogen is taken away from water (or a hydrated organic compound) and transferred to the oxidized intermediate (such as dehydroascorbic acid). If this be the case, then the Krasnovsky reaction, compared to the Hill reaction, lacks not only the enzymatic step leading to free oxygen, but also one of the two photochemical steps. It was suggested (20, p. 161) that such a two-step photochemical hydrogen transfer provides one of two possible explanations of why two (or more) quanta would be needed to move one hydrogen atom from H_2O to CO_2 . This brings us again to the subject of the quantum yield of photosynthesis, which is outside the scope of this review.

LITERATURE CITED

1. Heitz, E., *Ber. deut. botan. Ges.*, **54**, 362 (1936); *Planta*, **26**, 134 (1936)
2. Doutreligne, J., *Proc. Acad. Sci. Amsterdam*, **38**, 886 (1935)
3. Frey-Wyssling, A., *Discussions Faraday Soc.*, No. 5, 130 (1949); Frey-Wyssling, A., and Steinman, E., *Biochim. et Biophys. Acta*, **2**, 254 (1948)
4. Geitler, L., *Planta*, **26**, 463 (1936)
5. Vatter, A. (Unpublished data)
6. Weier, E., *Am. J. Botany*, **23**, 645 (1936); **25**, 501 (1938); *Botan. Rev.*, **4**, 497 (1938)
7. Kausche, G. A., and Ruska, H., *Naturwissenschaften*, **28**, 303 (1940)
8. Roberts, E. A., *Bull. Torrey Botan. Club*, **67**, 535 (1940); *Am. J. Botany*, **29**, 10 (1942)
9. Granick, S., and Porter, K. R., *Am. J. Botany*, **34**, 545 (1947)
10. Algera, L., Beijer, I. J., Iterson, W. van, Karstens, W. K., and Thung, J. H., *Biochim. et Biophys. Acta*, **1**, 517 (1947)
11. Frey-Wyssling, A., and Mühlethaler, K., *Vierteljahrsschrift Naturforsch. Ges. Zürich*, **94**(3) (1949)
12. Frey-Wyssling, A., *Discussions Faraday Soc.*, No. 5, 130 (1949)
13. Wyckoff, R. W. G., *Electron Microscopy* (Interscience Publishers, Inc., New York, N. Y., 248 pp., 1949)
14. Pardee, A. B., Schachman, M. K., and Stanier, R. Y., *Nature*, **169**, 282 (1952); Stanier, R. Y. (Personal communication)
15. Hubert, B., *Rec. trav. botan. néerland.*, **32**, 323 (1936)
16. Frey-Wyssling, A., *Submikroskopische Morphologie des Protoplasmas und seiner Derivate* (Gebr. Borntraeger Verlag, Berlin, Germany, 317 pp., 1938); *Sub-microscopic Morphology of Protoplasm and Its Derivatives* (Elsevier Publishing Co., London, England, 255 pp., 1948)
17. Menke, W., *Protoplasma*, **22**, 56 (1934); *Kolloid Z.*, **85**, 256 (1938)
18. Takashima, S., *Nature* (In press)
19. Hanson, E. A., *Rec. trav. néerland.*, **36**, 180 (1939)
20. Rabinowitch, E. I., *Photosynthesis*, **I**, Chap. 1-20, (Interscience Publishers, Inc., New York, N. Y., 1945); **IIA**, Chap. 21-30, (1951); **IIB** (In press)
21. Timm, E., *Z. Botan.*, **38**, 1 (1943)
22. Sisakyan, N. M., Bezinger, E. N., and Kuvaeva, E. B., *Compt. rend. acad. sci. U.R.S.S.*, **74**, 385 (1950)
23. Osipova, O. P., and Timofeeva, I. V., *Compt. rend. acad. sci. U.R.S.S.*, **74**, 979 (1950)
24. Osipova, O. P., and Timofeeva, I. V., *Compt. rend. (Doklady) acad. sci. U.R.S.S.*, **80**, 449 (1950)
25. Neish, A. C., *Biochem. J.*, **33**, 293, 300 (1939)
26. Hill, R., and Lehmann, H., *Biochem. J.*, **35**, 1190 (1941)
27. Noack, K., and Liebich, H., *Naturwissenschaften*, **29**, 302 (1941)
28. Veher, A. S., *Biokhimiya*, **12**, 196 (1947)
29. Menke, W., *Z. physiol. Chem.*, **263**, 100, 104 (1940)
30. Burr, G. O., *Proc. Roy. Soc. (London)*, [B]**120**, 42 (1936)
31. Mommaerts, W. F. H., *Am. J. Botany*, **25**, 561 (1938)
32. Neish, A. C., *Biochem. J.*, **33**, 293, 300 (1939)
33. Steemann-Nielsen, E., and Kristiansen, J., *Physiol. plantarum*, **2**, 325 (1949)
34. Day, R., and Franklin, J., *Science*, **104**, 363 (1945)

35. Bradfield, J. R. G., *Nature*, **159**, 467 (1947)
36. Waygood, E. R., and Clendenning, K. A., *Can. J. Research*, [C]**28**, 673 (1950)
37. Vennesland, B., Gollub, M. C., and Speck, J. F., *J. Biol. Chem.*, **178**, 301 (1949)
38. Arnon, D. I., *Nature*, **167**, 1008 (1951); Arnon, D. I. (Paper presented before Am. Soc. Plant Physiol., Minneapolis, Minn., September, 1951)
39. Frenkel, A. W., *Plant Physiol.*, **16**, 654 (1941)
- 39a. Boichenko, E. A., *Biokhimiya*, **13**, 88 (1948)
40. Hill, R., and Scarisbrick, R., *New Phytologist* (In press)
41. Rosenberg, A. Y., and Ducet, G., *Compt. rend.*, **229**, 391 (1949)
42. Davenport, H. E., and Hill, R., *Proc. Roy. Soc. (London)* (In press); Hill, R., *Symposia Soc. Exptl. Biol.*, **5**, 222 (1951)
43. Holt, A. S., *U. S. Atomic Energy Commission Document*, ORNL-752 (1950)
44. Arnon, D. I., *Plant Physiol.*, **24**, 1 (1948); *Nature*, **162**, 341 (1949)
45. Warburg, O., and Lüttgens, W., *Biokhimiya*, **11**, 303 (1946)
46. Bonner, J., and Wildman, S. G., *Arch. Biochem.*, **10**, 497 (1946)
47. Krossing, G., *Biochem. Z.*, **305**, 359 (1940)
48. Conn, E. E., Vennesland, B., and Kraemer, L. M., *Arch. Biochem.*, **23**, 179 (1949)
49. Sisakyan, N. M., and Kobyakova, A. M., *Biokhimiya*, **13**, 88 (1948)
50. Nezgovorov, S., *Compt. rend. acad. sci. U.R.S.S.*, **29**, 620 (1941)
51. Emerson, R., and Lewis, C. M., *Am. J. Botany*, **30**, 165 (1943)
52. Emerson, R., and Lewis, C. M., *J. Gen. Physiol.*, **25**, 579 (1942)
53. Dutton, H. J., and Manning, W. M., *Am. J. Botany*, **28**, 516 (1941)
54. Tanada, T., *Am. J. Botany*, **38**, 276 (1951)
55. Haxo, F. T., and Blinks, L. R., *J. Gen. Physiol.*, **33**, 389 (1950)
56. Blinks, L. R. (Personal communication)
57. Thomas, J. B., *Biochim. et Biophys. Acta*, **5**, 186 (1950)
58. Dutton, M. J., Manning, W. M., and Duggar, B. M., *J. Phys. Chem.*, **47**, 308 (1943)
59. Vermeulen, D., Wassink, E. C., and Reman, G. H., *Enzymologia*, **4**, 254 (1937)
60. Manning, W. M., and Strain, H. H., *J. Biol. Chem.*, **151**, 1 (1943)
61. Duysens, L. N. M., *Nature*, **168**, 548 (1951)
62. French, C. S., and Young, V. K., *J. Gen. Physiol.* (In press)
63. Livingston, R. (Personal communication)
64. Emerson, R., and Lewis, C. M., *Am. J. Botany*, **30**, 165 (1943); *J. Gen. Physiol.*, **25**, 579 (1942)
65. Ehrmantraut, H. C., and Rabinowitch, E., *Arch. Biochem.* (In press)
66. Livingston, R., *2nd Ann. Rept. ONR Research Project 059,028* (1949)
67. Vavilov, S. I., quoted from Shpolskij, E. A., *Uspekhi Fiz. Nauk*, **40**, 341 (1950)
68. Perrin, J., *2me Conseil Solvay*, 322 (Gauthiers-Villars, Paris, France, 1925); Perrin, F., *Ann. Phys.*, **17**, 283 (1932)
69. Vavilov, S. I., and Feofilov, P. P., *Compt. rend. acad. sci. U.R.S.S.*, **34**, 220 (1940); Vavilov, S. I., *J. Exptl. Theoret. Phys. (U.R.S.S.)*, **7**, 141 (1943); *Compt. rend. acad. sci. U.R.S.S.*, **42**, 331 (1944)
70. Arnold, W., and Oppenheimer, J. R., *J. Gen. Physiol.*, **33**, 423 (1950)
71. Förster, T., *Naturwissenschaften*, **33**, 166 (1946); *Z. Naturforsch.*, **2b**, 174 (1947); *Ann. Physik*, **2**, 55 (1948)
72. Franck, J., and Teller, E., *J. Chem. Phys.*, **6**, 861 (1938)
73. Gaffron, H., and Wohl, K., *Naturwissenschaften*, **24**, 81, 102 (1936)

74. Scheibe, G., *Z. Elektrochem.*, **47**, 73 (1941); **49**, 372, 383 (1943)
75. Jacobs, E. E. (Unpublished data)
76. Hill, R., *Proc. Roy. Soc. (London)*, [B]**127**, 192 (1939); Hill, R., and Scarisbrick, R., *Proc. Roy. Soc. (London)*, [B]**129**, 238 (1940)
77. Holt, A. S., and French, C. S., *Arch. Biochem.*, **19**, 429 (1948)
78. Clendenning, K. A., and Ehrmantraut, H. C., *Arch. Biochem.*, **29**, 337 (1951)
79. Warburg, O., and Lüttgens, W., *Biokhimiya*, **11**, 303 (1946)
80. Aronoff, S., *Plant Physiol.*, **21**, 393 (1946)
81. Holt, A. S., Smith, R. F., and French, C. S., *Plant Physiol.*, **26**, 164 (1951)
82. French, C. S., and Milner, H. W., *Symposia Soc. Exptl. Biol.*, **5**, 232 (1951); Milner, H. W., Lawrence, N. S., and French, C. S., *Science* **111**, 653 (1950); Milner, H. W., Koenig, M. L. G., and Lawrence, N. S., *Arch. Biochem.*, **28**, 185 (1950); Milner, H. W., French, C. S., Koenig, M. L. G. and Lawrence, N. S., *Arch. Biochem.*, **28**, 193 (1950)
83. Kumm, T., and French, C. S., *Am. J. Botany*, **32**, 291 (1945)
84. Clendenning, K. A., and Gorham, P. R., *Can. J. Research*, [C]**28**, 78, 102, 114 (1950)
85. French, C. S., Holt, A. S., Powell, R. D., and Anson, M. L., *Science*, **103**, 462 (1946)
86. Arnold, W., and Oppenheimer, J. R., *J. Gen. Physiol.*, **33**, 423 (1950)
87. French, C. S., Anson, M. L. and Holt, A. S. (Unpublished data)
88. Arnon, D. I., and Whatley, F. R., *Arch. Biochem.*, **23**, 141 (1949)
89. Gorham, P. R., and Clendenning, K. A., *Can. J. Research*, [C]**28**, 513 (1950)
90. Holt, A. S., Smith, R. F., and French, C. S., *Plant Physiol.*, **26**, 164 (1951)
91. Holt, A. S., and French, C. S., *Arch. Biochem.*, **9**, 25 (1946)
92. Gurevich, A. A., *Compt. rend. acad. sci. U.R.S.S.*, **55**, 263 (1947)
93. Holt, A. S., and French, C. S., *Arch. Biochem.*, **19**, 368 (1948)
94. Mehler, A. H., *Arch. Biochem. Biophys.*, **33**, 65 (1951)
95. Holt, A. S., *U. S. Atomic Energy Commission Document*, ORNL-752 (1950)
96. French, C. S., and Holt, A. S., *Arch. Biochem.*, **19**, 368 (1949)
97. Ochoa, S., *Symposia Soc. Exptl. Biol.*, **5**, 29 (1951); Vishniac, W., and Ochoa, S., *Federation Proc.* (In press)
98. Tolmach, L. J., *Arch. Biochem.*, **33**, 120 (1951)
99. Kok, B., *Enzymologia*, **13**, 1 (1948); *Biochim. et Biophys. Acta*, **3**, 625 (1949)
100. Veen, R. van der, *Physiol. plantarum*, **2**, 217 (1949)
101. Gaffron, H., Fager, E. W., and Rosenberg, G. L., *Symposia Soc. Exptl. Biol.*, **5**, 262 (1951)
102. Warburg, O., Geleik, H., and Briese, K., *Z. Naturforsch.*, **6b**, 134, 285, 417 (1951)
103. Rabinowitch, E., *Ann. Rev. Phys. Chem.*, **2**, 361 (1951)
104. Boichenko, E. A., *Compt. rend. acad. sci. U.R.S.S.*, **38**, 181 (1944)
105. Boichenko, E. A., *Biokhimiya*, **12**, 196 (1948)
106. Franck, J., *Revs. Modern Phys.*, **17**, 112 (1945)
107. Brown, A. H., and Franck, J., *Arch. Biochem.*, **16**, 55 (1948)
108. Hill, R., Davenport, H. E., and Whatley, F. R., *Proc. Roy. Soc. (London)* (In press)
109. Spikes, J. D., Lumry, R., Eyring, H., and Wayrynen, R. E., *Arch. Biochem.*, **28**, 48 (1950)
110. Holt, A. S., Brooks, I. A., and Arnold, W., *J. Gen. Physiol.*, **34**, 627 (1951)

111. Weller, S., and Franck, J., *J. Phys. Chem.*, **45**, 1360 (1941)
112. Macdowall, F. D. H., *Plant Physiol.*, **24**, 462 (1949)
113. Gaffron, H., *J. Gen. Physiol.*, **26**, 195, 241 (1942)
114. Emerson, R., and Arnold, W., *J. Gen. Physiol.*, **15**, 391 (1932); **16**, 191 (1932)
115. Davis, E. A., *Science*, **108**, 110 (1948); *Am. J. Botany* (In press)
116. Fan, C. S., Stauffer, J. F., and Umbreit, W. W., *J. Gen. Physiol.*, **27**, 15 (1943)
117. Shiau, Y. G., and Franck, J., *Arch. Biochem.*, **14**, 253 (1947)
118. Livingston, R., and Ke, C. L., *J. Am. Chem. Soc.*, **72**, 905 (1950); Livingston, R., *2nd Ann. Rept. ONR Research Project 059,028* (1949)
120. Timiriazev, K. A., *Proc. Roy. Soc. (London)*, [B]**72**, 424 (1903)
121. Kuhn, R., and Winterstein, A., *Ber.*, **66**, 1741 (1933)
122. Rothemund, P., *Cold Spring Harbor Symposia Quant. Biol.*, **3**, 71 (1935); Albers, V. M., Knorr, M. V., and Rothemund, P., *Phys. Rev.*, **47**, 198 (1935)
123. Kosobutskaja, L. M., and Krasnovsky, A. A., *Compt. rend. acad. sci. U.R.S.S.*, **84**, 103 (1950)
124. Krasnovsky, A. A., *Compt. rend. acad. sci. U.R.S.S.*, **60**, 421 (1948)
125. Krasnovsky, A. A., *Compt. rend. acad. sci. U.R.S.S.*, **61**, 91 (1948)
126. Krasnovsky, A. A., and Brin, G. P., *Compt. rend. Acad. sci. U.R.S.S.*, **67**, 325 (1949)
127. Krasnovsky, A. A., Brin, G. P., and Vojnovskaja, K. K., *Compt. rend. acad. sci. U.R.S.S.*, **69**, 393 (1949)
128. Krasnovsky, A. A., and Brin, G. P., *Compt. rend. acad. sci. U.R.S.S.*, **73**, 1239 (1950)
129. Evstigneev, V. B., and Gavrilova, V. A., *Compt. rend. acad. sci. U.R.S.S.*, **74**, 781 (1950)
130. Holt, A. S. (Unpublished data)
131. Ghosh, J. C., and Sen-Gupta, S. B., *J. Indian Chem. Soc.*, **11**, 65 (1934)
132. Livingston, R., Sickie, D., and Uchiyama, A., *J. Phys. Chem.*, **5**, 755 (1947)
133. Livingston, R., and Pariser, R., *J. Am. Chem. Soc.*, **70**, 1510 (1948)
134. Rabinowitch, E., and Weiss, J., *Proc. Roy. Soc. (London)*, [A]**162**, 251 (1937)
135. Ashkinazi, M. S., Glikman, T. S., and DaIn, B. J., *Compt. rend. acad. sci. U.R.S.S.*, **73**, 743 (1950)
136. Ashkinazi, M. S., and DaIn, B. J., *Compt. rend. acad. sci. U.R.S.S.*, **74**, 385 (1951)
137. Linschitz, H., *J. Am. Chem. Soc.* (In press)
138. Kachan, A. A., and DaIn, B. J., *Compt. rend. acad. sci. U.R.S.S.*, **80**, 619 (1951)
139. Porret, D., and Rabinowitch, E., *Nature*, **140**, 321 (1937)
140. Livingston, R., *J. Phys. Chem.*, **45**, 1312 (1941); McBrady, J. J., and Livingston, R., *J. Phys. Chem.*, **52**, 662 (1948); Livingston, R., *2nd Ann. Rept., ONR Research Project 059,028* (1949)

PHYSIOLOGY OF FLOWERING¹

BY ANTON LANG^{2,3}

*Kerckhoff Laboratories of Biology, California Institute of Technology,
Pasadena, California*

Flowering can be separated into the following major stages: (a) floral initiation (the differentiation of floral primordia); (b) floral organization (the differentiation of the individual flower parts); (c) floral maturation, consisting of several processes, some of them concurrent or overlapping (growth of the flower parts, differentiation of the sporogeneous tissues, meiosis, pollen and embryo sac development); and (d) anthesis.

In this review the physiology of all four stages has been considered. The treatment of the three later stages, however, has been combined and is limited to those changes which involve sporophytic tissues only and which are integrating elements of the flowering process. For this reason, the physiology of meiosis, the entire pollen physiology, and the development of flower pigmentation and of any accessory flower parts are excluded. Throughout the treatment, stress has been laid upon the mechanism of the developmental changes, that is, on those physiological and biochemical processes which actually initiate or control development. Work of a purely descriptive nature has been referred to only when it is apt to shed some light on mechanisms as well. The review is therefore by no means a complete survey of the literature, even of the most recent vintage.

FLORAL INITIATION

Floral initiation and the later stages of flowering.—Of the various stages of flowering, floral initiation is by far the most fundamental one, for it marks the actual switch from vegetative to reproductive development. Most of the work on the physiology of flowering has been concerned with this stage. This is so even though in many cases some later stage (anthesis or the appearance of visible buds) has been observed, for the variations in these stages have been but projections of variations in the onset of floral initiation. In this connection, however, an important methodological question must be raised. Variations in the onset of floral initiation frequently persist unchanged throughout the following stages of flower development; but the initiation of floral primordia and their subsequent fate are still separate phenomena and may show a different dependence on one and the same condition.

¹ The survey of the literature pertaining to this review was concluded in December, 1951.

² Lalor Foundation Fellow.

³ The author is greatly indebted to Dr. James Bonner, Dr. A. W. Galston, Dr. K. C. Hamner, and Dr. J. L. Liverman for numerous valuable suggestions in preparing this review, and to these and many other colleagues for permission to use unpublished data.

Furthermore, the time of floral initiation depends on the rate of the preceding vegetative growth, and conditions which influence this rate may cause differences in the time of flower formation without having affected initiation in a specific manner. It therefore becomes imperative to use a criterion which permits clean separation between specific and nonspecific effects in floral initiation. Such a criterion is the relative amount of the preceding vegetative growth, as expressed by the number of leaves (or leaf pairs or whorls) preceding the first flower. This approach was first used in a consistent manner by Purvis (185, 190) in *Secale*; it is readily applicable to any plant with terminal flowers or with a clearly demarcated terminal inflorescence and also to many plants with a systemic habit of flowering, for example, *Gossypium* (121). Presence or absence of differences in the leaf numbers tells us whether initiation has been affected specifically or not, regardless of whether or not there are differences in the time of appearance of floral primordia, buds, or open flowers.

Photoperiodism and vernalization.—In numerous plants floral initiation shows a highly specific dependence on certain environmental conditions. These conditions may be quite different from those which are favorable for growth and other vegetative functions of the plant. The bulk of our information on the physiology of floral initiation has been derived from the study of such cases, for in plants lacking a specific environmental control of flowering it is extremely difficult to modify this process experimentally and thus make it accessible to analysis. The two conditions which most frequently control floral initiation in a specific manner are daylength and low temperature. The control of physiological processes by daylength or photoperiod—of which flower formation is but one case, although the most common and the most spectacular one—is called photoperiodism. The low-temperature requirement in annual herbaceous plants, in which it has been studied most thoroughly, has been termed vernalization.

The basic facts of photoperiodism and vernalization are well known. The terms and abbreviations which follow will be used in this review.

(a) Long-day plants (LDP): plants in which flowers are formed only in daylengths exceeding a definite value or in which flower formation is promoted in such daylengths.

(b) Short-day plants (SDP): plants in which flowers are formed only in daylengths below a definite value, or in which flower formation is promoted in such daylengths.

(c) Inductive daylength: any daylength permitting or accelerating flower formation.

(d) Noninductive daylength: any daylength preventing flower formation or retarding it maximally.

(e) Critical daylength: the daylength value separating the inductive and the noninductive ranges of photoperiod.

(f) Day-neutral or indeterminate plants: plants in which flower formation is not specifically dependent on daylength.

(g) Biennial plants: plants with a qualitative low-temperature requirement in flower formation.

(h) Winter annual plants: plants with a quantitative low-temperature requirement.

(i) Summer annual plants: plants with no low-temperature requirement.

Recently, a reclassification of plants with respect to their daylength dependence has been suggested, involving new names for all of the photo-

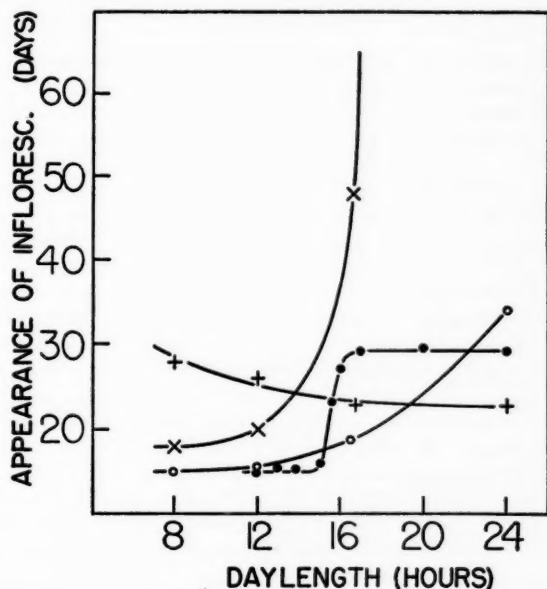


FIG. 1. The photoperiodic response of several species and strains of *Xanthium*. +—+, *X. spinosum* (day-neutral plant; the slight promotion of flowering in high daylengths is probably unspecific). x—x, one strain of *X. strumarium* (short-day plant with qualitative daylength response) and ●—●, another strain of *X. strumarium* (short-day plant; quantitative daylength response; pronounced critical daylength). o—o, *X. orientale* (short-day plant; quantitative response; no critical daylength). Data after Lang (118) and Lona (135).

periodic types (44). It is difficult, however, to see the point of such a proposal. The accepted terms are not only highly suggestive of what they describe, but also convey all that can be conveyed in the present state of our knowledge. It is essential, however, to use the recognized terms with greater accuracy. Thus, definitions of LDP and SDP, which are based on absolute or on relative specifications of daylength, are bound to be imperfect, since the inductive ranges of the two types overlap and critical daylength is a charac-

ter which is specific for every single species or variety. Even the definitions given above, which are unequivocal where they are applicable, are not quite all-inclusive. In some plants, any change of daylength throughout the entire range will promote or retard flower formation. These plants, thus, do not possess a pronounced critical daylength (see Fig. 1), and are not adequately covered in a classification based on this concept. This question will be returned to later (see p. 284).

PHOTOPERIODISM

The floral stimulus arising in photoinduction.—Both daylength and low temperature have a pronouncedly inductive mode of action. The effect appears with some delay and, in fact, frequently after the causative condition is no longer operative. In vernalization this is frequently the rule, for during the actual low-temperature treatment (the thermal induction) the growth and development of the plant may be completely suspended. In photoperiodism, flowering usually occurs most rapidly with continuous inductive treatment, but some flowering response can be obtained with inductive periods far too short to permit any flower formation in the course of the actual treatment. This aftereffect of limited periods of inductive treatment is called photoperiodic induction. In addition, the effect of photoinduction in LDP is not reduced by interruptions with periods of short-day conditions (79, 123, 132, 169, 208), and it is possible to obtain flower formation in an LDP by "fractional induction," that is, by exposing the plant to an induction period insufficient to cause a flowering response and repeating this subinductive treatment after a period of short-day conditions.

Thus, both in low-temperature and in daylength action we have to distinguish between the immediate action of the inductive conditions and the subsequent changes which result in actual floral initiation, and we have to try to separate these different changes. This is particularly urgent in photoperiodism for it seems that the opposite conditions of long and short days result, in different plants, in one and the same response, i.e. flower formation. To understand the daylength control of floral initiation, we must start by establishing whether or not the final changes which are responsible for this process are identical in LDP and SDP.

It has been known for some time that floral initiation in LDP and in SDP depends on the photoperiodic treatment of the leaves and that it is immaterial whether or not the sites of the actual response, the growing points, are subjected to the inductive conditions (17, 28, 31, 78, 81, 82, 94, 96, 108, 130, 133, 159, 160, 229). Also, the effect of temperature on the flowering response is the same if either entire plants or the leaf blades alone are kept at different temperatures during photoinduction (176, 178), whereas differential temperature treatment of the petioles or the stem tips becomes effective only at much lower temperatures (19). The immediate action of daylength takes place in the leaves and the effect is transmitted to the growing points.

Two basically different possibilities can be visualized: (a) the inductive daylength promotes floral initiation; and (b) the noninductive daylength inhibits it. The former alternative implies that an LDP or an SDP is incapable of floral initiation unless it is photoinduced. The latter alternative implies that the plant is potentially capable of floral initiation under any daylength, but that initiation is secondarily suppressed by noninductive daylength conditions. The evidence summarized below shows clearly that a flower-promoting effect of the inductive daylength is an essential factor in photoinduction. First, favorable temperature conditions during induction promote floral initiation, unfavorable temperatures delay or suppress it (see p. 275). Second, if photoinduction is limited to part of a plant, flowers can also be formed (under certain conditions) in the noninduced parts. In extreme cases, photoinduction of a single leaf is sufficient, even if all other leaves of the plant are maintained on the noninductive conditions (81, 82, 94, 229). Finally, if a plant is photoinduced and then is grafted to an individual kept on the noninductive conditions, the latter plant will also initiate flowers (Table I. The graft partner capable of flower formation is called donor, the partner not capable of flower formation by itself is called receptor).

All these findings show that under the influence of the inductive daylength conditions, changes take place in the plant which actively promote floral initiation. In many cases, a single leaf is sufficient as the donor in a graft. The leaf can be detached from a plant and can be photoinduced while growing as a cutting [Lona (140)]. This fact shows that all the processes of floral initiation which are under immediate daylength control can be completed within the leaves—and the leaves alone. We can conclude that floral initiation in LDP and in SDP is determined by a floral stimulus which is generated in the leaves under the influence of photoinduction and is then translocated to the growing points.

The question whether there exists a comparable, that is a transmissible, flower-inhibiting effect of the noninductive daylength conditions, will be considered later. At present we want to ask ourselves what the relationship is between the floral stimuli of LDP and SDP. Attempts to identify the stimuli chemically have met with failure (see p. 287); therefore, only an indirect approach is possible, again by way of grafting. Grafts can be made not only between induced and noninduced plants of the same response type, but with equal ease between plants belonging to the opposite response types. In such experiments (see Table I) it becomes evident that the stimulus from an LDP can also cause flower formation in an SDP and vice versa. The stimuli of the two photoperiodic types are interchangeable.

This interchangeability, however, is not yet a conclusive proof of identity. It is unlikely that we are dealing with two entirely unrelated and independent stimuli which have the same physiological activity; but it is conceivable that there are two independent complementary stimuli or that one stimulus is formed in two stages. In that case we can visualize that in LDP

TABLE I
FLOWER-INDUCTION GRAFTS†

Receptor		Donor			References
Short-day plant in long-day conditions	Long-day plant in short-day conditions	Short-day plant in short-day conditions	Long-day plant in long-day conditions	Day-neutral plant	
A. Intravarietal grafts					
<i>Perilla frutescens</i> var. <i>nankinensis</i> <i>Xanthium</i> spp. <i>Glycine soja</i> (soybean)	<i>Petunia hybrida</i>	<i>Perilla frutescens</i> var. <i>nankinensis</i> <i>Xanthium</i> spp. <i>Glycine soja</i> (same strains as receptors)	<i>Petunia hybrida</i>		29, 41 81, 196 113, 196 196
B. Intraspecific grafts					
<i>Nicotiana tabacum</i> var. Maryland Mammoth <i>Glycine soja</i> var. Biloxi, Peking <i>Gossypium hirsutum</i> ssp. <i>typicum</i> ,** <i>marigalante</i> <i>Xanthium strumarium</i> **				<i>Nicotiana tabacum</i> var. <i>Sam-sun</i> etc. <i>Glycine soja</i> var. <i>Agate</i> , <i>Batorawka</i> * <i>Gossypium hirsutum</i> ssp. <i>typicum</i> † <i>Xanthium strumarium</i> ‡	153, 161 69, 99 121 157
C. Interspecific grafts					
<i>Helianthus tuberosus</i> <i>Nicotiana tabacum</i> var. Maryland Mammoth <i>Gossypium davidsonii</i> <i>Gossypium raimondii</i>		<i>Nicotiana glauca</i>		<i>Helianthus annuus</i> <i>Gossypium hirsutum</i> † <i>Gossypium hirsutum</i> ‡	29 153 121 121
D. Intergeneric grafts					
<i>Nicotiana tabacum</i> var. Maryland Mammoth <i>Xanthium saccharatum</i>	<i>Hyoscyamus niger</i> (annual strain)	<i>Nicotiana tabacum</i> var. Maryland Mammoth	<i>Hyoscyamus niger</i> (annual strain)	<i>Callistephus sinensis</i> Δ	153 125 217

† The table lists the grafts in which daylength-dependent plants kept on noninductive conditions (the receptors) were brought to flower formation by graft union to plants capable of flower formation (the donors). As donor, either a day-neutral plant, or a daylength-dependent plant photoinduced either before or after the grafting can be used.

* Little daylength effect in floral initiation.

** Short-day strains.

† Daylength dependent.

‡ Doubtful classification.

Δ Classification doubtful.

the first, and in SDP the second, of these stimuli or stages is produced only under the inductive conditions, whereas in either case the other stimulus or stage is independent of daylength. To check these possibilities one must establish whether donors of the one response type cause floral initiation in receptors of the other type only if they are induced themselves. Such experiments have so far been made only in one case, using *Nicotiana tabacum* var. Maryland Mammoth as SDP and *N. silvestris* and *Hyoscyamus niger* as LDP (105, 121, 125). With *Hyoscyamus* as receptor, flower formation occurred only with short-day treated donors. In *N. silvestris*, some response was ob-

TABLE II

EFFECT OF INDUCED AND NONINDUCED DONORS UPON RECEPTORS OF THE OPPOSITE PHOTOPERIODIC RESPONSE TYPE
(After 105, 121, and 125)

Receptor	Donor	Flowering response*	
		Donor under inductive conditions	Donor under non-inductive conditions
<i>Hyoscyamus niger</i> (LDP)	<i>Nicotiana tabacum</i> var. Maryland Mammoth (SDP)	+	0
<i>Nicotiana silvestris</i> (LDP)	<i>Nicotiana tabacum</i> var. Maryland Mammoth	(+)†	(+)†
<i>Nicotiana tabacum</i> var. Maryland Mammoth	<i>Hyoscyamus niger</i>	(+)	0
<i>Nicotiana tabacum</i> var. Maryland Mammoth	<i>Nicotiana silvestris</i>	+	0

* Flowering response: + Flowers initiated in all grafts
(+) Flowers initiated in part of grafts
0 No flower initiation

† Perhaps unspecific effect, see (125).

served both with induced and with noninduced donors, but the entire effect may be nonspecific (see 125). Maryland Mammoth receptors flowered only when the *Hyoscyamus* and *N. silvestris* donors were maintained on long days (see Table II). While an extension of the experiments is desirable, the results indicate full identity of the floral stimuli of LDP and SDP.

Light and darkness in the photoperiodic response of long-day plants.—The foregoing discussion shows that the immediate flower-controlling effect of daylength is localized in the leaves and that it results in the formation of a floral stimulus which is alike in LDP and SDP. The difference between the two response types must be sought in those changes which photoinduction causes to take place in the leaves. Daylength or, as it was described by Garner

and Allard, the daily light-dark ratio, is not an elementary environmental condition, like light alone, temperature, humidity, etc., but is a composite of light and darkness. The first task in the elucidation of photoperiodic responses will be the disentangling of the respective roles of the two conditions. The principal material available for this task stems from two types of approaches: the use of light-dark cycles of different total length and the use of differential experimental conditions in the light and in the dark periods. To establish the individual significance of the two periods they must be varied separately, but as long as one works with the natural 24-hr. cycles the length of the two periods can be varied only simultaneously. Therefore, one must change the total length of the cycle or subject the plants to one set of experimental conditions in the light periods and to another set in the dark periods. Another approach which has proven to be very valuable is the application of small amounts of light in the dark periods, given either as extended periods of illumination with very low light intensities, or as short-time interruptions with light.

In the case of LDP, both indirect and direct evidence indicates that an essential factor in their photoperiodic response is an inhibitory effect of long dark periods, an effect which under short-day conditions prevents the formation of the floral stimulus.

The indirect evidence follows. (a) LDP flower in continuous light and no kind of light-dark alternation has been found to be superior to the continuous light regime (43, 79, 114, 119, 123, 132, 164). Thus, dark periods do not have any indispensable positive part in the formation of the floral stimulus in LDP. (b) LDP stay vegetative if short light periods are combined with long dark periods. If, however, short light periods are given together with short dark periods, flower formation takes place (3, 121, 208).⁴ The critical factor in short-day action is not the shortness of the light periods but the length of the dark periods. (c) In most LDP flowering response and temperature seem to be negatively related. Floral initiation is promoted and the critical daylength is lowered as the temperature is decreased (109, 123, 197), the decisive role in this effect being played by the temperature of the dark periods (114, 123, 174). The inhibitory effect of dark periods seems to increase with increasing temperature and floral initiation is suppressed in shorter periods of time. The seemingly reverse temperature relationship which has been observed in a few LDP [*Rudbeckia bicolor*, *Bouvardia humboldtii* (167, 197, 210)] need not be a true exception to the rule, for the inverse relationship between temperature and floral initiation in LDP may hold only for a certain range of temperature and may break down if these limits are transgressed (132).

The direct evidence is based on work done with the LDP, *Hyoscyamus niger*. Flowers in this species are promptly initiated under short-day condi-

⁴ Under "short" and "long" light and dark periods, any periods are understood which are shorter or longer, respectively, than the light or dark period at the critical daylength of the plant in question.

tions if the plants are kept continuously defoliated; the rate of floral initiation in defoliated plants is the same in long and short days (117, 122, 123). This demonstrates not only that the formation of the floral stimulus is actively suppressed in short days, but also that the inhibitory action is seated in the leaves, while the axis tissues are free from it. It is also clear that under short-day conditions the leaves are capable of preventing the formation of the stimulus in the axis as well. In other words, the inhibitory action extends over some distance.

Now arises the question of what the exact function of light is in the formation of the floral stimulus in LDP. Does it only remove the adverse effects of dark periods or does it, in addition, have some positive, promotive function of its own? At first glance, the latter alternative seems to be the right one. Floral initiation can be induced in LDP by supplementing the light periods of short days with light of very low intensities (48, 64, 230, 231) or by interrupting the dark periods of short days with brief periods of light (15, 23, 49, 169, 180, 214, 215). The energies necessary for complete annihilation of the effect of long dark periods lie, although apparently varying within rather wide limits in different plants, between 100 and 1000 f.c.min. (foot candles \times minutes). A significant effect may be obtained with 10 f.c.min. On the other hand, the light energies required for the effectiveness of the light periods fall in the range of photosynthetically active light. In sugar beets (*Beta vulgaris*), for example, the minimum is about 700 f.c. of continuous light (169) or approximately 1×10^6 f.c.min. per 24 hr. It appears that we are dealing with two distinct light actions, one which promotes the formation of the floral stimulus directly and requires high amounts of light energy and another which removes the inhibitory effects of darkness and requires small amounts of light energy.

However, the relations of light to the formation of the floral stimulus in LDP are apparently more complex. *Hyoscyamus* forms flowers upon defoliation not only under short-day conditions, but also in continuous darkness (117, 122, 123). Thus, if no inhibition is present, the formation of the stimulus is not dependent on light at all; light periods are necessary only because this process is normally suppressed in dark periods. It is true that to date a comparable effect of defoliation has not been observed in other LDP (42, 123, 125, 132). But in these cases the defoliated plants failed to produce flowers not only in short days or in continuous darkness, but also under long-day conditions. Therefore, these negative results do not invalidate the positive ones which have been secured with *Hyoscyamus*. It seems that either the generation of the floral stimulus itself in most LDP takes place only in the leaves or that to carry out this function (or perhaps, in some cases, to carry on a sufficient amount of growth, which is of course the prerequisite for the production of any new structures) these plants need a continuous supply of some material which is formed only in light and in the leaves. *Hyoscyamus* is unique only in that it is capable of carrying out these functions at the expense of stored material alone. It is important to note that

this plant also forms flowers upon defoliation only after having attained a certain size and having produced a well developed storage root. Intact plants are sensitive to photoinduction at a much earlier age.

The specific action of light in the photoperiodic control of floral initiation in LDP is, then, the counteraction of the inhibitory effect of dark periods in the formation of the floral stimulus. This effect is accomplished by small amounts of light energy. High-intensity light has only some preparatory role and does not enter directly into the formation of the floral stimulus.

This latter concept is supported by several additional facts. If *Hyoscyamus* is grown on 48-hr. cycles, floral initiation occurs with shorter periods of light per cycle than it does in 24-hr. cycles (9 versus 11 hr.), although the dark periods are naturally much longer (50). In the LDP *Spinacia* (spinach), flower formation can be obtained in individuals raised in total darkness (on sugar-containing media) (70). It is possible that the inhibitory effect which appears in the dark periods is ultimately dependent on the normal development and functioning of the leaves, that is, on the presence of high-intensity light periods.

The question which remains now is how the counteracting effect of the low-intensity light is achieved. In considering this question, two most essential facts must be taken into account: (a) while LDP remain vegetative on short-days, they form flowers with long light periods even if the dark periods are simultaneously extended by a much greater factor (3, 50, 132, 208); and (b) as noted earlier (p. 268), an interruption of the photoinduction of an LDP does not reduce its effectiveness. It thus seems that the inhibitory action of dark periods is limited to the immediately adjacent light periods. This may be caused partly by the fact, discussed in the preceding paragraph, that the effectiveness of the dark periods is not unlimited, but declines if the periods become too long. However, fractional induction could be explained on this basis only if the maximum of the dark-period effect were already reached at the critical daylength. Since the dark-period effect is increased by higher temperature, this does not seem to be the case. Two assumptions can be made: (a) a dark period tends to destroy the changes which have taken place in the preceding light period, but if the light period is extended beyond a certain limit, further changes take place which are insensitive to the dark-period effect; (b) some adverse effect of the preceding dark period must be eliminated in the course of a light period before the formation of the floral stimulus can set in. The first alternative implies that the formation of the floral stimulus proceeds in two stages and that the second cannot be entered until the first has attained a certain level (208). The second alternative implies that the floral stimulus itself is insensitive to the dark-period effect from the beginning and is being produced to the extent that the dark-period effect has been sufficiently reduced. This alternative seems more plausible. It is also supported by the observation that supplementary low-intensity light (see above) is more effective in promoting floral initiation if given prior to the high-intensity light period than if given afterwards (64). This may mean that the initial low-intensity light removes the dark-period effect

and thus enables the plant to utilize fully the high-intensity light. The evidence, however, is not yet conclusive. If the light action follows the dark action, it must be assumed, because of the defoliation experiments in *Hyoscyamus*, that the dark action involves the production of a transmissible, inhibitory material. This possibility has not been studied so far. As long as it has not been proven one may consider that the inhibiting action of darkness is localized entirely within the leaves and that the distance action which is evident in *Hyoscyamus* is caused by the diversion of some material necessary for the production of the floral stimulus from the axis tissues to the leaves (cf. 123). An inhibitory effect of short-day leaves has also been noted in other LDP in experiments with localized photoinduction (38, 39, 229); but this may be interpreted either way and, in addition, may be caused not by an inhibition of the formation of the stimulus, but of its translocation from long-day leaves to growing points (see p. 282). As long as these questions are not settled, the exact mode of interaction of light and darkness in the photoperiodic response of LDP remains an open question.

Light and darkness in short-day plants.—The significance of light and darkness in SDP becomes evident from the following results. (a) SDP initiate flowers only if they receive light-dark cycles with sufficiently long dark periods. If the dark periods are reduced below a certain limit the flowering response will decline and will ultimately fail; however, the response is also reduced if the light periods are reduced, even if the dark periods are maintained at an optimum level (see data in 3, 79, 81, 164, 198). Furthermore, if differential treatment is applied in the light and the dark periods of photoinduction (see p. 272), the response is affected in a similar manner: it is promoted by favorable conditions (favorable temperatures) and is reduced or suppressed by unfavorable ones (low or excessively high temperature, application of narcotics) (81, 87, 143, 145, 176). Both light and dark periods are evidently needed for the formation of the floral stimulus in SDP. (b) In some SDP [*Xanthium* (79); *Chenopodium amaranticolor* (139)] a single photoinductive cycle may be sufficient to cause floral initiation. In this case, the light period has to precede the dark period (79). Thus, light periods of sufficient length are needed to render inductive dark periods effective. (c) If the light periods are kept at a constant, optimal level and the dark periods are extended, the flowering response is reduced (3, 198). The beneficial effect of a light period seems to be limited; when it is used up, the dark period effect, which has already built up, apparently begins to deteriorate. This deterioration must be rather slow, for the decline of the response with extension of the dark periods is gradual (3, 198), and complete failure occurs, if at all, only with very long dark periods [in *Kalanchoë blossfeldiana*, for example, 88–112 hr (98)].⁵

⁵ It seems that in some SDP the effect of the dark periods does not decline as the periods are extended but continues to increase, although apparently at a slow rate. *Perilla* plants, which will not respond to a single optimal 24-hr. induction cycle, form flowers if exposed to a single long dark period (130 hr. or more). If, however, the plants are given several 24-hr. cycles, the total number of dark hours required for photoinduction may be as low as 36 (139).

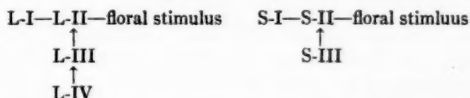
(d) If an SDP can be photoinduced with a single light-dark cycle, there is no upper limit to the light period; but in SDP that require more than one induction cycle, extension of the light periods leads to a rapid decrease of the flowering response (3, 79, 198). Also, fractional induction seems to be not possible in SDP, at least not without loss in effectiveness (14, 98, 121, 143). Thus, while light is required to make the following dark period effective, the effect of a dark period is reduced and can be nullified in the following light period. In other words, we are dealing in SDP with two antagonistic light effects. The formation of the floral stimulus depends on two sets of changes, one requiring light energy, the other inhibited by light. The light-inhibited changes cannot set in before the light-requiring changes have become effective; but they must be, in their turn, protected from too long light periods. The floral stimulus is produced or persists in SDP only if the plants are subjected to regular alternations of appropriate light and dark periods.

The inhibitory action of light on SDP is accomplished by the same minute quantities of light as is the low-intensity light action on LDP. These quantities may be given as low-intensity supplementary light (64, 230, 231) or as brief light interruptions in the dark periods (79, 81, 84, 181, 192, 206, 214, 215). This light action is very similar to the low-intensity light action on LDP, but its effect with regard to floral initiation is just the opposite.

The minimum duration of the light periods and the minimum amounts of light which are required to make them effective, show an amazing variation between species. Some species need light periods comparable to the minimum light periods of some LDP. Such plants have sometimes been considered as a separate photoperiodic group [intermediate or middle-day plants (2, 164)]; but the demarcation between these and the usual SDP is a gradual one. Several typical SDP have minimum light periods between two and five hours (164), and the light intensities required in the light periods of SDP are comparable to those needed in the light periods of LDP (cf. 17, 79, 145). In *Kalanchoë blossfeldiana*, however, as little as one second of daylight proves sufficient to secure a flowering response which is not greatly inferior to the optimal response obtainable [Harder & Gümmer (88)]. In *Xanthium* and in *Perilla*, flower formation can be induced by transferring the plants to continuous darkness (79, 139). In *Perilla* this treatment is effective even if it is preceded by an extended light period of very low light intensity [Lona (139)]. Extension of the light periods in *Kalanchoë* results in an increase of the response only after periods of approximately 30 min. have been reached. These remarkable facts suggest that the promotive light action in floral initiation of SDP may be composed of two different effects: one which requires only small amounts of light energy and another which becomes effective only with rather high amounts of light energy. The high-intensity light effect can apparently be dissociated from the actual processes of floral initiation, at least in some short-day species, and can be replaced by storage material. Similar to the high-intensity light action of LDP, this effect is of a preparatory nature. This idea is again supported by the observations that *Perilla*

plants respond to the dark treatment only after they have reached a certain age and that the effectiveness of the extremely short light periods in *Kalanchoë* is consistent only in plants which are in good vegetative condition. The promotive low-energy light effect, in turn, seems to have some immediate function in the formation of the floral stimulus, for if the one-second light periods in *Kalanchoë* are omitted and the plants are kept in continuous darkness, they stay strictly vegetative (88).

Chemical changes involved in the formation of the floral stimulus.—The analysis of the photoperiodic responses of LDP and SDP enables us to distinguish several well defined partial processes in the formation of the floral stimulus. We can distinguish four such processes in LDP: a preparatory high-intensity light process (L-I); a light-independent process which results in the actual appearance of the stimulus (L-II); a dark process antagonistic to L-II (L-III); and a low-intensity light process antagonistic, in its turn, to L-III (L-IV). The following partial processes are well established in SDP: a light process (S-I) and a dark process (S-II), both of which are necessary for the stimulus to be produced; and a low-intensity light process antagonistic to S-II (S-III). If antagonistic relationships are expressed by arrows, the sequence of the processes can be written as follows:



As discussed above, S-I consists quite likely of a preparatory, high-intensity light process comparable to L-I and of a low-energy light process which is directly involved in the production of the floral stimulus. L-II may have to be subdivided into two separate processes (see p. 274), only the first of which would be subject to the action of L-III.

The separation of the whole process of the formation of the floral stimulus in LDP and SDP into a series of partial processes provides us with a basis for biochemical approaches to the problem. Before this separation had been achieved, there was practically no way to establish if any chemical changes following photoinduction were related to floral initiation in a causal or in an incidental manner. The extensive studies of such changes which were carried out in the earlier period of research in photoperiodism, or were carried out later without taking into account the recognition of the partial processes, have yielded next to nothing towards a biochemical understanding of the photoperiodic responses.

The possibility and necessity of separating photoperiodic responses into partial processes have been recognized only in the course of the last 10 or 12 years, beginning with the work of Hamner & Bonner (81) and Hamner (79) with SDP and of Lang & Melchers (123) with LDP. In view of this short period of time it is understandable that the amount of conclusive biochemical information on the individual partial processes is still very limited. What we

do know concerns the high-intensity light process of LDP (L-I), the promotive light process of SDP (S-I) and the inhibitory dark process of the former (L-III). In addition, we have some evidence that the auxin level in the leaves plays some important part in the photoperiodic induction, at least in SDP. This question will be considered in a separate section.

The high-intensity light processes of both photoperiodic types seem to be identical with photosynthesis or to be closely tied in with it. This fact is suggested in the first place by the order of magnitude of the light intensities which are required for these processes, and is supported by the following evidence: (a) *Hyoscyamus* plants initiate flowers under noninductive conditions if supplied with sugar (123); and (b) in *Xanthium*, floral initiation can be induced by a single dark period if the plants, instead of being subjected to a high-intensity light period, are fed with sugar (12). The experiments with *Spinacia* (p. 274) and with defoliated *Chenopodium* (p. 285) may also be cited in this connection.

If the high-intensity light processes of LDP and SDP are identical with photosynthesis, their function is evidently restricted to securing the substrates which furnish the energy required in the formation of the floral stimulus and their relationship to this latter process would be indirect—in fact, rather remote. This is in agreement with the findings, discussed earlier, that in some plants the high-intensity light effect can be separated from the actual formation of the stimulus and can be replaced by storage material. In LDP this nonspecificity is particularly evident, for the effectiveness of the inhibitory dark process (L-III) seems also to depend ultimately on the presence of adequate light periods (see p. 274). L-I would seem to supply the substrates for any reactions involved in flower formation, whether promotive or inhibitive.

As to what part of the energy metabolism is involved in the generation of the floral stimulus, we have only one experimental indication. In *Xanthium*, the light periods may be replaced not only by feeding sugar, but also by organic acids of the Krebs cycle (131). An additional supply of these acids during the light periods of photoinduction increases the flowering response (106). Thus, the essential factor may be the transformation of pyruvic acid rather than the preceding glycolytic sequence.

As to the light which promotes floral initiation in SDP (low-energy effect), we have to date again only one indication of the direction in which its effect might be sought. It has been found that photoinduction is effective in SDP only if the atmosphere in which the plants are maintained during the light periods contains CO_2 (177). This seems to be a further proof that the high-intensity light process is identical with photosynthesis. But since in *Kalanchoë* only one second of light is required, this is probably not the sole explanation. In *Kalanchoë*, too, CO_2 is necessary for photoinduction. Moreover, in localized inductive treatment the short-day parts of the plant must be supplied with CO_2 directly; the presence of CO_2 over large long-day areas is of no avail (86). This necessity suggests that some CO_2 fixation not identi-

cal with photosynthesis may be involved in the formation of the floral stimulus and that to carry out this fixation the plants must receive at least a small amount of light. It would be most interesting to know whether CO_2 must be present during the one-second light periods themselves or whether it might be given shortly afterwards. Whether the effectiveness of organic acids has a special meaning in this connection also remains to be seen.

Process L-III is tied in with oxidative reactions, for floral initiation in LDP can be caused under short-day conditions by keeping the plants during the dark periods (or part thereof) in an atmosphere of nitrogen (48, 152, 238).

Auxin and photoperiodic induction.—The role of auxin in the photoperiodic induction of SDP is basically clear. First, flower formation by SDP can be suppressed under short-day conditions by treating the plants with auxin or synthetic growth-regulating substances (13, 90, 131, 141, 201, 218) and can be induced under noninductive conditions by treating with auxin antagonists (8, 131) or with ethylene chlorhydrin (105), an agent which also seems to lower the auxin content of plants (cf. 158, 213). Thus, floral initiation depends on a reduction of the physiological auxin level in the plant. Second, leaves of a SDP which were treated during photoinduction with an auxin are not effective if used as donors to receptor plants maintained on long days (13). Thus, the crucial factor seems to be the auxin level in the leaves during photoinduction, and the effect of high auxin levels seems to consist in an inhibition of the formation of the floral stimulus. Third, the effect of auxin on photoinduction is most pronounced if the application is made at the beginning of the dark period (9). If the application is made following the dark period (9, 131) or the end of the photoinductive treatment (131, 201), the effect is much smaller or nonexistent. Thus, the auxin level seems to be specifically associated with the functioning of the dark-period process of SDP (S-II) or with its immediate outcome, and the effect of inductive dark periods seems to involve a lowering of the auxin level in the leaves. The later stages of flower development seem to require an increase in the auxin supply, for in *Xanthium*, antiauxin-induced inflorescence primordia may fail to develop unless the plants are treated later with auxin (131).

In what manner inductive dark periods affect the auxin level in the leaves, and in what exact manner this level is involved in the formation of the floral stimulus, is entirely a matter for speculation. An essential step in the elucidation of this problem would be a direct determination of the changes in the auxin level of a leaf in the course of an inductive dark period and of photoinduction in general. To date, only some comparisons of the auxin content in short-day and in long-day treated plants are available. They do indicate that the amount of extractable auxin may be less under short-day conditions (9, 12, 233, 237); however, wherever in these determinations the relationship between the activity and the dilution of the extracts was checked, it was not found to be linear. These results, therefore, are not reliable. The apparent reason for the lack of linearity lies in the fact that the leaves of numerous plants contain some auxin inhibitor. Its presence not only prevents auxin

determinations by the classical methods, but may also complicate the entire issue, for the physiological auxin level in a tissue may depend, not only on the amount of auxin itself, but also on the amount of auxin antagonist present. It is to be hoped that the use of modern separation techniques in auxin analysis (103, 129) will help us to resolve this difficulty.

The question as to what role, if any, auxin has in the photoperiodic control of floral initiation in LDP is an entirely open one. Most LDP grow in the vegetative state as rosettes. Stem elongation coincides with floral initiation and may, in fact, be slightly ahead of it, even in LDP in which the further development of flowers may proceed without stem elongation [certain *Rudbeckia* species (77, 91, 167)]. A close interrelationship between the two processes and thus between floral initiation and auxin is indicated. Occasionally, however, stem elongation has been observed in rosette-type, long-day plants without any flower formation, although the conditions causing this occurrence are not clear (123), and in some LDP [species of *Urtica*, *Anagallis*, *Circaea*, etc. (cf. 44, 137)] stem elongation is quite independent of floral initiation and takes place both in long- and in short-day conditions. The results of auxin applications and of experimental treatments which decrease the auxin content of plants (X-ray and ultraviolet irradiation) are not less controversial. In *Silene armeria*, auxin treatment seems to be capable of inducing floral initiation under noninductive conditions (131). In *Hyoscyamus* the results have so far been negative, but the periods of treatment may have been too short (49). Under long-day conditions, flower formation was delayed by auxin application in one LDP (*Calendula officinalis*) and was speeded by ultraviolet irradiation in two other presumptive LDP (*Statice bonduelli* and *Linum*), but remained unaffected in numerous other cases (58, 59). In *Hordeum* (barley) the number of flowers (spikelets) initiated, also under long-days, was increased both by x-ray irradiation and by the application of low auxin dosages, although it was decreased by higher dosages (127). However, in this case there is no proof that we are concerned with a specific effect in floral initiation.⁶

The absorption of the photoperiodic light energy.—Since various light actions are involved in the photoperiodic responses of LDP and SDP, there arises the problem of the nature of the system mediating the absorption of the light energy and the immediate changes caused by this energy. We have at present some information about the action spectra of the low-intensity light process of LDP (L-IV), the inhibitory light process of SDP (S-III), and one piece of evidence about the nature of the last-named process. Comparable studies on the high-intensity light processes in both photoperiodic types are lacking. Such studies should yield material information about the nature and the specificity of these processes, but the high energies required make them a technically difficult job.

⁶ A general discussion of the effects of auxin in flower formation is given by Bonner & Bandurski in another article in this volume (pp. 59-86) and also in Bonner & Liverman (11).

Our information about the action spectra of the two low-intensity light processes comes from the work of Borthwick, Hendricks, and Parker. This work has been reviewed in Volume I of the *Annual Review of Plant Physiology* (179; see also 20, 21). It has revealed that we are dealing with a specific light-absorbing system hitherto not noted in higher plants; that the system is identical in LDP and SDP (15, 180, 181); and that the same system mediates the light energy in several other morphogenetic effects of light, namely in the control of stem elongation (15) and of leaf growth (182). In the earlier work of these authors, the extent to which the spectra might have been modified by screening effects of other pigments was in some doubt, but recently the same spectrum (for the control of stem elongation) has been demonstrated in an albino mutant of *Hordeum* free of any of the major plant pigments (16). Such complete absence of screening would be hard to understand if the system which absorbs the photoperiodic light energy were localized in the chloroplasts. This result, therefore, suggests that it is localized in the cytoplasm.

The action spectra obtained by Borthwick and co-workers show absorption throughout the entire visible spectrum, but with a pronounced maximum in the red and a second, much smaller maximum, in the blue. This finding agrees in an over-all fashion with the results of most of the earlier work done in the field (104, 107, 223, 230, 231, 232). None of the early work had, however, been sufficiently quantitative to reveal the difference between the photoperiodic action spectrum and that of photosynthesis and the implication had, therefore, been that the two were identical. Funke alone has maintained that in addition to those plants in which the photoperiodic response is mainly determined by red light, there are three other groups: one responding equally well to red and blue, one responding only to red plus blue, and one responding to blue alone (66, 67, 68). Since Funke's technique was not very satisfactory, these claims were open to some doubt. It comes, therefore, somewhat as a surprise that recently Wassink *et al.* (225), using fully reliable equipment (cf. 224), found in an LDP, a variety of *Brassica napus oleifera*, a photoperiodic action spectrum entirely different from that of Borthwick and co-workers and similar to that of Funke's fourth type, namely, principal effectiveness in blue and violet, and, in addition, in the near infrared (below 0.95μ). It is not entirely clear, however, whether the *Brassica* spectrum relates to floral initiation, for the leaf numbers which are given in one instance show comparatively small differences and do not corroborate the clear-cut superiority of the blue-violet range indicated by the dates of bud appearance. But the spectrum as such remains very interesting, particularly the effectiveness of infrared. Effectiveness of infrared (1.0 – 2.5μ) is also reported in the SDP *Perilla* (166).

Borthwick and co-workers conclude from the study of their action spectra that the absorbing system is probably an open-chain tetrapyrrol pigment, such as a phycocyanin. Direct attempts at identification have not yet succeeded (183). In the absence of further indications as to the nature of the

pigment, its identification promises to be a very tedious task, for a rough estimate, based on the amount of characteristic absorption in the region of maximum effectiveness in the albino *Hordeum* seedlings, indicates that the pigment is present in exceedingly small quantities (16).

The small quantity of the pigment may account, however, for the low-intensity character of this photoperiodic light action. The system which absorbs the light energy for this action will be saturated at low intensities and any light exceeding these intensities will be wasted for the photoperiodic response.

The information about the nature of the inhibitory light process of SDP stems from work of Harder *et al.* (92) on *Kalanchoë*. Harder and co-workers found that the effect of light interruptions in the dark periods of photoinduction is entirely independent of temperature. This result shows that either the entire process S-III is a single photochemical reaction or that any biochemical reactions associated with the light reaction require a comparatively long time to become effective and therefore are not affected by the temperature conditions prevailing in the short periods of the application of light.

The movement of the floral stimulus.—The floral stimulus is normally transmitted through leaf, petiole, and stem tissue, but it can also pass through root tissue (35, 211). The translocation is entirely nonpolar (for example, 28, 31, 81, 137, 201); the stimulus may, for example, move down one branch of a plant and up another. The movement out of the leaves can apparently take place through the mesophyll, for it is not affected by severing the midrib at the base of the leaf blade (33). In petioles and stems, the stimulus probably moves in the phloem. This probability is indicated in the first place by two observations. First, girdling, steam treatment, localized low temperature, or narcotics treatment of stems and petioles all reduce or interrupt transmission (19, 36, 69, 228). The stimulus evidently moves in living cells. Second, in plants with opposite leaves the stimulus stays preferentially in the stem sector adjacent to the leaf, although this may be apparent only with low amounts of the stimulus (nonoptimal photoinduction) or only in the early stages of inflorescence development (83, 85, 95, 142). Since dyes introduced into the conducting system exhibit the same behavior the movement evidently proceeds in the vascular bundles.

The principal evidence for the phloem transport of the stimulus, however, is furnished by another fact. Both in localized photoinduction and in grafts, the flowering response of the noninduced part or partner is greatly reduced by the presence of leaves (37, 38, 39, 81, 89, 93, 98, 121, 128, 131, 133, 136, 137, 163, 165, 170, 201, 211). This inhibitory effect is present both in LDP and in SDP, although in the former it is generally less pronounced. It may be ascribed to the generation by the noninductive leaves of a solute stream opposed to the solute stream coming from the induced leaves. This means, of course, that the floral stimulus is carried along in the stream of photosynthates moving in the sieve tubes. This explanation is very strongly suggested by the following facts: (a) the inhibitory effect is exerted only by

mature leaves, that is, leaves capable of efficient photosynthesis (for example, 165); (b) it is exerted exclusively by leaves located between the source of the stimulus and the receiving bud (or in such cases where the induced and noninduced leaves are located at equal distances from the bud and no other buds are present in the plant) (38, 39, 83, 201, etc.); and (c) the inhibition is enhanced if the youngest leaves on the receptor shoot are removed, that is, if the effectiveness of the region attracting the solute stream is reduced (81, 128, 131, 165). The inhibitory effect of long-day leaves in SDP can be simulated by supplying defoliated, long-day parts of the plant with sugar solutions, thus generating an artificial solute stream opposed to the solute stream moving from the induced leaves (40). In the SDP *Perilla*, the inhibitory effect of long-day leaves is reduced if the light intensity given to these leaves is decreased (40); in the LDP *Urtica pilulifera*, the inhibitory effect of short-day leaves disappears if the light periods given to the short-day parts are reduced to 1 to 3 hr. per day (137). In either case, the leaves apparently are no longer capable of generating an effective solute stream. In *Urtica* and also in the SDP *Kalanchoë* (201), but apparently not in *Perilla* (37, 165) nor in the LDP *Beta* (211), inhibition is also exerted by leaves kept in continuous darkness. Such leaves appear to act as sinks which intercept the solute stream and sidetrack the floral stimulus.

The inhibitory effect of leaves on the translocation of the floral stimulus can be demonstrated only with leaves kept under the noninductive daylength conditions. It is, however, very probably a normally occurring phenomenon. Photoinduction of a single leaf, and even part of a leaf, in the absence of inhibiting leaves may give a flowering response equal to that obtainable with photoinduction of the entire intact plant (18). If the stimulus were formed in each leaf in great excess, there should be an inverse proportionality between the minimal number of inductive cycles and the number of leaves left on a plant. In reality, it takes the same number of cycles to induce an intact plant and a plant defoliated to one leaf. It thus seems as if the relative effectiveness of photoinduction decreases with increasing size of the treated area. One reason for this relationship is probably that the efficiency of different leaves in the production of the floral stimulus is not the same. The sensitivity of a leaf to photoinduction increases until full expansion and then becomes gradually smaller (18, 160, 170); both older and immature leaves apparently produce less stimulus than mature, but younger leaves. However, another, and quite probably the principal, reason is that leaves which are closer to the growing point interfere with the translocation of solutes from more distant leaves so that the floral stimulus formed in the latter is not efficiently transmitted to the growing points.

The only attempt to estimate the translocation rate of the floral stimulus has yielded values approximating 2 cm in 24 hr. in stems and 0.5 cm in 24 hr. in roots (34, 35). These values are considerably below the translocation rates estimated for solute movement in sieve tubes. It is, however, uncertain whether the observed rates were optimal, since in the experiments the length

of the transport route (down and up a whole stem, split lengthwise) may have been out of proportion to the size of the supplying area (a single leaf).

The general mechanism of photoperiodism.—The study of LDP and SDP, as reviewed in the foregoing sections, provides us with a fairly definite idea of the general mechanism of daylength action in floral initiation. The action consists in adjusting the balance of several individual processes participating in floral initiation, some of them having a promotive and some an inhibitory character. This is an important result in itself, for such a situation is by no means self-evident. One could have thought that in LDP, floral initiation depended on a single light-requiring process, in SDP, on a single light-inhibited process, and that periods of light and darkness respectively merely delayed the attainment of the necessary effect, but did not interfere with the effect already accumulated. In such a case, only one of the two component conditions of daylength, either light or darkness, would be the controlling factor, with the other acting as a mere passive interruption. The term photoperiodism, in fact, would not be justified. But in the daylength effect on floral initiation, light and darkness both play an active part and their actions must be properly timed for flower formation to occur. This daylength effect thus contains a definite element of periodicity. In LDP, however, the action of dark periods with regard to floral initiation is a purely inhibitory one. An actual alternation of light and dark periods is therefore necessary for (or promotive of) floral initiation in SDP alone. One can say that the real photoperiodic response in SDP is the induction or promotion of floral initiation by the inductive daylength conditions, whereas in LDP the response is the suppression or retardation of floral initiation by the noninductive conditions.

On the basis of these considerations, one can arrive at new and all-inclusive definitions of LDP and SDP. LDP are plants in which the daily dark periods inhibit or delay flower formation and SDP are plants in which the daily dark periods induce or accelerate flower formation. These definitions also cover those plants which lack a pronounced critical daylength (see p. 267) and therefore eluded classifications based on the critical daylength concept. It must only be borne in mind that in some plants daily dark periods of any duration will affect flower formation, whereas in the majority the dark periods do not become effective unless in excess of a certain minimum value.

These definitions have the additional advantage of reflecting the situation in nature. The shortest daylengths encountered anywhere in the world in the course of the growing season are still quite in excess of the minimal light periods required for floral initiation in SDP. The only exceptions are the so-called intermediate plants (see p. 276) which require both a comparatively long light period and a comparatively long dark period. These plants, therefore, flower only in a very narrow range of natural photoperiods. But LDP, too, are capable of flower formation with quite short light periods, provided these are not accompanied by long dark periods (see

p. 272). Thus, both in SDP and in LDP under natural conditions the factor which determines whether and when flower formation occurs is the length of the daily dark period.

The daylength-controlled stages of floral initiation are passed in the leaves. This does not mean that other plant parts are entirely incapable of responding to photoinduction or of bringing about floral initiation. We have seen that completely defoliated *Hyoscyamus* plants form flowers (p. 272). Defoliated plants of *Chenopodium amaranticolor* (139) and *Xanthium* (126) form flowers under short-day conditions. Such cases also show that the relative strength of the different processes can vary in different parts of a plant. In *Hyoscyamus* the inhibitory dark process (L-III) is limited to leaf tissue. A comparable, although more quantitative, situation seems to exist in *Chenopodium* with regard to the inhibitory light process (S-III). If defoliated plants of *Chenopodium* are fed with sugar, they become capable of forming flowers under long-day conditions [Lona (138)]. If enough substrate is available, apparently the process S-II becomes effective in stem tissue even in the presence of long light periods. However, if a single leaf is left on the *Hyoscyamus*, *Chenopodium*, or *Xanthium* plants, floral initiation (under inductive conditions) occurs faster than in completely defoliated individuals. There is no doubt that in intact plants photoperiodic response and floral initiation are determined by the activity of the leaves.

We now must ask if our knowledge of the general mechanism of daylength action enables us to formulate a more detailed interpretation that will cover LDP and SDP at the same time. In either response type, we have recognized a series of processes controlling the formation of a floral stimulus. The stimulus is the same in both types. Therefore, its production probably follows one and the same pathway in LDP and in SDP, and a process which participates in the formation of the stimulus in a direct and promotive manner in LDP should also be present in the SDP, and conversely. Furthermore, the systems which mediate the light energy in the low-intensity light process of LDP (L-IV) and in the inhibitory light process of SDP (S-III) are also identical and it is therefore very probable that these two processes are identical in turn and that their seemingly opposite effect with regard to floral initiation is based on a quantitative rather than a qualitative type of difference.

We might make, for example, the following assumptions: (a) the formation of the floral stimulus depends on the presence of the proper auxin level in the leaves; (b) the auxin level decreases in the course of dark periods; and (c) in SDP the appropriate auxin level is reached only after an extended dark period, whereas in an LDP the auxin level is lowered by such a dark period to ineffectiveness. If this interpretation is correct, the processes L-III and S-II and the processes L-IV and S-III would be identical. The two former would consist in a reduction of the auxin level. Processes L-I and S-I would also be identical, and process L-II would consist in the actual formation of the floral stimulus in SDP as well as in LDP.

$$L-I = S-I-L-II-\text{floral stimulus}$$

$$L-III = S-II$$

$$L-IV = S-III$$

It must be emphasized, however, that the above interpretation is a mere conjecture, given as a more tangible illustration of the kind of relationships we may have to look for rather than as an hypothesis which is established on specific experimental evidence. Our insight into the role of auxin in photoperiodic induction and floral initiation is far too insufficient to draw definite conclusions, and we do not know of an auxin-increasing light effect as assumed above. Quite generally we must admit that our knowledge of the partial processes involved in the formation of the floral stimulus in LDP and SDP is not yet detailed enough and that much more experimental work will be needed before we will be in a position to make precise comparisons between the individual processes of the two response types.

We also cannot be quite certain to have recognized all major processes participating in the photoperiodic responses. While those processes of which we do know will account in an over-all fashion for all aspects of the behavior of LDP and SDP, some features in the kinetics of their responses are still puzzling. The dark process of SDP (S-II) can be nullified if the dark periods are interrupted by very small amounts of light; yet, its effect evidently survives the very much longer light period of the next inductive cycle. One may assume that the effect of the dark process builds up, not in a linear fashion, but slowly at first and then with greatly increasing rapidity; but it remains a matter for amazement that an effect which is abolished by one minute of light after 9 hr. of darkness can, after 15 hr. of darkness, be nullified only by light periods of somewhere between 14 and 19 hr. duration (data after 84 and 198).

This extraordinary change in light sensitivity can be accounted for in two basically different ways. One can once more postulate that several consecutive changes take place in the course of inductive dark periods and that their products differ in the degree of light-sensitivity. This assumption has been made by Hamner (79, 80) who thinks, that after the beginning of the dark period changes take place which after a definite duration of the dark period reach a threshold value, and that then other changes set in, the result of which is much less light-sensitive than the result of the former.

One can, however, also assume that the efficiency of the light itself changes in the course of a dark period. This idea has been introduced by Bünning (22 to 27), and Bünning has made it the basis of a general hypothesis of photoperiodism, suggesting that the changes in light efficiency are related to the so-called endogenous daily fluctuations of activity which are known to occur in plants. The evidence which Bünning adduces in favor of this general explanation cannot be considered convincing, two principal objections being that the course of the endogenous periodicity has hardly been studied in plants with a clear-cut daylength dependence of flower formation

and that the endogenous periodicity occurs also in day-neutral plants and therefore cannot be the direct basis of photoperiodic responses but can, at best, contribute an additional element to their mechanism. The fundamental idea, however, remains, namely, that it may not be at all the course of some light-sensitive process involved in floral initiation that changes in a quantitative or qualitative manner in the course of the dark period, but that it is the relative effectiveness of light which changes in the course of this process. This idea is supported by one piece of information, obtained in an LDP. In *Hyoscyamus* plants grown on 48-hr. cycles the effect of light interruptions of the dark periods shows two maxima in the course of the period, separated by a period of little effectiveness (50).

Nature and action of the floral stimulus.—Our discussion of the photoperiodic responses on LDP and SDP has been based on the concept that photoinduction results in the formation of a transmissible floral stimulus. This idea was generally accepted after the correlative nature of the day-length action was clearly demonstrated. It was assumed that the stimulus is a specific substance, a flower hormone or "florigen" [Cailahān (28, 30, 31, 32), Moškov (161)]. Recently, however, several authors have argued against this idea (57, 138, 139, 141, 193). It has not yet been possible, in spite of some extensive and careful attempts, to extract an active material from photoinduced plants and to introduce it into noninduced test individuals (81, 153). Positive effects which have been reported in a few cases either proved irreproducible (10) or so slight that a conclusive confirmation is urgently required (195). In view of this failure it is argued that the specific processes of flower formation take place in the growing points and that the effect of day-length consists not in the formation of flower-promoting substances under the inductive conditions, but in the formation of flower-inhibiting substances under the noninductive conditions. Some of the authors identify these inhibitory substances with auxin (57, 193, 201) and suggest that photoinduction involves the production of auxin antagonists (201).

Several considerations bear on this controversy.

(a) Nonextractability is no conclusive proof against the existence of a flower hormone. It appears that cellular continuity is indispensable for the transmission of the floral stimulus because transmission in grafts occurs only after tissue union between donor and receptor has taken place (163, 228). [Some claims to the contrary were either not confirmed in later studies (81, 161) or are dubious for lack of exacting controls (71).] This fact may be based on the mode of translocation of the stimulus (along with the solute stream in sieve tubes [see p. 282]) and may render both the isolation of the hormone and its reintroduction into test plants virtually impossible.

(b) The evidence presented on p. 269 shows beyond doubt that some changes which promote floral initiation actively and which can be communicated to noninduced plant parts or to other plants do arise during photoinduction. Consequently, only two things can be assumed: (1) that these changes are not of a specific nature, but consist in the production of greater

amounts or of different ratios of the gross assimilates; and (2) that in addition to the flower-promoting changes arising under inductive conditions, there exists some flower-inhibiting material which is formed under noninductive conditions and prevents the promoting material from functioning.

(c) The possibility of fractional induction (see p. 268) is a very strong argument in favor of the specificity of the flower-promoting changes, for if the changes were such as mentioned in (a) 2, they could hardly persist and accumulate through extended periods of noninductive conditions. Fractional induction is possible only in LDP, but since the floral stimuli of LDP and SDP are identical, this argument also holds for the latter.

(d) In contrast to the evidence which can be adduced in favor of the existence of transmissible flower-promoting effects, the evidence in favor of transmissible flower-inhibiting substances is very poor. There is no doubt that the daylength control of flower formation involves inhibitory effects, but these effects seem to be directed, not against their functioning, but against the formation of flower-promoting substances. The main argument brought forward for the existence of flower-inhibiting substances antagonistic to the floral stimulus is the inhibitory action of noninduced leaves (see p. 282). This very action, however, can be accounted for in terms of translocation and is thus the least specific one that could be imagined. If it were based on the production of an inhibitory material, it would be impossible to see why it is limited to leaves located between the source of the floral stimulus and the responding bud. Actually, in *Kalanchoë*, even leaves located between an induced leaf and the bud are not inhibitive, or are but little so, unless inserted on the same orthostichy (93). There are at present only two cases in which the formation of some transmissible flower-inhibiting material appears possible, namely that of *Chenopodium* described on p. 285 and that of *Hyoscyamus* described on p. 272. In *Chenopodium*, intact (nondefoliated) plants do not form flowers under long-day conditions even if fed with sugar; thus the sugar effect evident in leafless individuals appears to be suppressed when leaves are present (138). In *Hyoscyamus*, under short-day conditions, the leaves seem to suppress the formation of the floral stimulus in the axis tissues. However, neither case is entirely conclusive, for we may again be dealing with translocation phenomena. In *Chenopodium*, the solute stream proceeding from mature leaves may prevent the comparatively small amounts of floral stimulus formed in the stem of sugar-supplied plants from reaching the growing point; in *Hyoscyamus*, the effect may be the result of the diversion of some material from the axis into the leaves (see p. 274).

(e) The idea that auxin is a flower-inhibiting agent is based on the inhibition of flower formation by applied auxin and its promotion by a lowered auxin level in the plant (see p. 279). But we have seen evidence that the auxin level enters into only one specific phase of photoinduction, at least in the case of SDP, and that this phase is once again concerned with the formation, and not with the functioning, of the floral stimulus. If the inhibitory effect of noninduced leaves were based on auxin production (201), one would

have to assume that the auxin is transported to the induced leaves. This transportation would in numerous cases necessitate an upward movement. Auxin, however, is known to move only downward.

(f) If photoinduction consisted in the production of an auxin antagonist which is transported to the growing points, floral initiation should be induced under noninductive conditions by antiauxin treatment of the growing points. This obvious experiment has apparently not been made, but experiments in which the whole plants were treated do not support the idea. Since, however, there is no critical evidence whatsoever that the auxin concentration in the growing points has something to do with floral initiation, the entire idea is questionable. Besides, it is difficult to comprehend why an anti-auxin could not be extracted from induced plants.

Two more general considerations also support the idea that the floral stimulus is a specific agent promoting floral initiation in a direct manner. One is the absence of any aftereffect of noninductive conditions. If these conditions acted by causing the formation of flower-inhibiting substances, one would expect that the longer a plant is maintained under the noninductive daylength, the more resistant it will be to photoinduction. In reality, the responsiveness to photoinduction increases either continuously (in such plants which ultimately flower even under extreme noninductive conditions) or until a final optimal level is reached.

The second consideration is based on the mode of action of daylength and it is also important for the general understanding of the role of daylength in the control of floral initiation. Floral initiation is an all-or-none event. Therefore, any factor or process which controls this event in a rather direct manner should likewise, have an all-or-none mode of action. If, on the contrary, a factor has a clearly quantitative effect, one can assume that some further processes intervene between its primary effect and floral initiation. Thus, if daylength were controlling floral initiation through the production of assimilates or of an auxin antagonist, whereas the specific processes of floral initiation took place in the growing points, one would expect photoinduction to have a quantitative effect. The effect of photoinduction in floral initiation is, however, pronouncedly qualitative, that is, of the all-or-none type. In *Hyoscyamus*, floral initiation takes place after the plant has received a definite number of photoinductive cycles, and cycles in excess of this number have no additional accelerating effect (123). Similarly, in short-day strains of *Gossypium hirsutum*, the first flowers are formed at approximately the same node, regardless of whether photoinduction was optimal or at the bare minimum of effectiveness (121). It appears that the floral stimulus is accumulated in the plant until a threshold concentration necessary for the differentiation of a floral primordium is attained; then the primordium is formed whether or not the production of the stimulus continues.

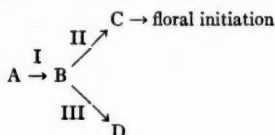
In summary, all available evidence is in agreement with the concept that the result of photoinduction is the appearance of a specific flower hormone which controls floral initiation in a direct and positive (promotive) manner.

Noninductive daylength conditions interfere with the formation and not with the functioning of the hormone.

VERNALIZATION AND ITS INTERRELATION WITH PHOTOPERIODISM

The mechanism of vernalization.—The changes which take place during the low-temperature treatment itself will be discussed first in the treatment of vernalization for reasons of clarity. The changes which follow the treatment will be taken up afterwards. The finding that vernalization is reversed by high temperature is of paramount importance for the understanding of the mechanism of the process [devernalization (61, 62, 124, 191, 212)]. Within the thermoinductive range—and within a certain range of treatment times—we find typical optimum curves for the temperature effect in vernalization (72, 120, 189, 212). Devernalization, in turn, becomes more effective as temperature is increased (124, 212). Over a particular temperature range, a temperature coefficient of more than three was found [Stout (212)]. This suggests that we are dealing with two antagonistic processes, one promotive and the other inhibitory with respect to ultimate floral initiation, and that both processes are of a chemical nature. Low temperature is apparently necessary because the promotive process is less accelerated than is the antagonist as temperature increases and may have a lower temperature optimum.

For more specific ideas the following three findings are significant: (a) as the low-temperature treatment progresses, the reversibility of vernalization in winter annuals (winter cereals) decreases and finally disappears entirely (5, 62, 76); (b) in biennial *Hyoscyamus niger*⁷ reversibility seems to disappear if the plants after the end of the cold treatment are kept for five or more days at room temperature (124); and (c) while with comparatively short times of treatment the effect of varying temperatures is different, results tend to equalize with prolonged treatment periods, so that in almost any temperature which is effective at all, the same level of vernalization seems to be finally reached (120, 189). These results are in agreement with an interpretation of vernalization suggested by Lang & Melchers (124), which can be formulated as follows:



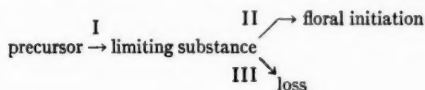
Floral initiation depends in some manner on the attainment of a definite condition, probably the formation of a specific chemical substance (C). C is produced by way of an intermediary, B. B, however, can be destroyed, in-

⁷ This plant occurs in annual and biennial strains, absence and presence of low-temperature requirement being determined by one gene (51, 148, 149).

activated, or sidetracked in some other way (to D), and thus be lost for the production of C. The three processes differ in their temperature dependence in such a way that in high temperatures all of B is diverted to D, whereas in lower temperatures at least part of B remains available for conversion to C. (A, B, and C or D need not be successive links in a straight chemical chain, but may be related in some indirect fashion; for example, B may be an enzyme catalyzing the synthesis of C from a precursor of its own.)

Chemical changes involved in vernalization.—Our insight into the biochemical changes which take place during the action of low temperature are based almost exclusively on the work of Purvis and Gregory on winter rye (*Secale cereale*). They find, first of all, that low temperature treatment of whole grains is effective right from the start of the treatment and occurs without a supply of any outside material (except oxygen, see below). Excised embryos, however, require the supply of an adequate kind and amount of sugar, and the first two low-temperature weeks (approximately) have no vernalizing effect (lag period) (74, 187, 188, 189; see also 110). Second, the sugar supply to excised embryos is particularly critical in the earlier period of the low-temperature treatment (188, 189). Third, the lag period disappears if the embryos are left attached to their endosperms for the first few days of the cold treatment, but it is not affected by any treatment given at room temperature (189). Fourth, cold treatment is effective only in the presence of some oxygen (63, 65, 75). Last, if the cold treatment is interrupted by periods at room temperature given under an atmosphere of nitrogen its effectiveness is lost (75). Similar interruptions given in air reduce the effect of the cold periods much less (provided the durations are not too long) (216); periods in nitrogen given at the vernalization temperature are similarly harmless (75).

The following conclusions can be drawn from the first three points above. First, the processes which take place during the cold treatment and which ultimately result in floral initiation depend on the presence of some substance which, in entire grains, is supplied from the endosperm, but which, in excised embryos, must be synthesized by the embryo itself. This synthesis depends on the presence of sugar, very probably as an energy substrate. Second, this substance persists in the embryo only at low temperature while in higher temperatures it is subject to some sort of loss. We thus find a situation basically identical with the one indicated by the temperature studies



and we are justified in assuming, tentatively, that the processes revealed in the two approaches are identical. The last two points indicate, in addition, that process II and possibly process I involve oxidative reactions, whereas process III seems to be fully effective in the absence of oxygen. A further

separation and characterization of the individual processes may be accomplished by the use of metabolic blocks. Certain enzyme poisons (arsenate and possibly fluoride) seem to inhibit vernalization in a specific way (46).

The cold-induced floral stimulus and its relationship to the daylength-controlled stimulus.—The temperature and metabolic studies carried out in vernalization show that a certain induced state must be reached in the plants for floral initiation to proceed and that the attainment of this state depends on the balance of several individual processes, some promotive and some antagonistic. This situation is the same as in photoperiodism, except of course that the environmental factor controlling the balance is not daylength but temperature.

In photoperiodism, the condition reached during induction was a transmissible floral stimulus—florigen. This fact was basically quite evident, since in the case of daylength the sites of perception and of response are clearly separated. In the action of low temperature, there is no comparable separation. The low-temperature effect in winter annuals can be perceived by the tissues of the embryo (see above and 71), the shoot tissue alone apparently being fully sufficient (186, 200), and in biennials by the stem tip alone (47, 54, 148, 149). However, if cold-treated individuals of biennial *Hyoscyamus* are grafted to noncold-treated ones, the latter are induced to form flowers [Melchers (148, 149)]. Thus, in the outcome of vernalization, too, a transmissible floral stimulus appears in the plant and we have to ask ourselves what the relationship of this stimulus is to florigen.

Some plants possess a low-temperature and a daylength (usually a long day) requirement simultaneously. If either of the requirements is of a strictly qualitative kind, as in biennial *Hyoscyamus*, floral initiation will take place only if thermal induction precedes photoinduction.* If biennial *Hyoscyamus* is grafted to *Nicotiana tabacum* var. Maryland Mammoth, a short-day plant which requires no low-temperature treatment, that is, a summer annual, and if the latter is prevented from flower formation by the non-inductive daylength (long-day) conditions, the receptor forms flowers only if the donor has been vernalized and is exposed to the inductive daylength (157; see 156, p. 161; see also 211). Thus, the vernalization changes must be completed before florigen can be formed.

The simplest possibility would then be that the stimulus transmitted from vernalized to nonvernalized *Hyoscyamus* plants is florigen, for it is conceivable that the immediate cold-induced state, while promoting the formation of florigen, remains localized in the cold-treated plants. However, grafting experiments reciprocal to those described in the last paragraph, with

* If the thermal induction is given at a temperature permitting some growth and at a daylength permitting some photoinduction, the plants may be thermo- and photoinduced simultaneously and may form flowers if afterwards kept under strictly noninductive daylength conditions (cf. 175). If, however, plants which had been raised under inductive daylength conditions are both cold-treated and afterwards continued on strict short-day conditions, no flower formation will take place.

nonvernalized biennials as receptors and daylength-dependent summer annuals as donors, prove that this explanation is not correct. Such grafts were first made by Melchers (150) using again biennial *Hyoscyamus* (as receptor) and Maryland Mammoth tobacco (as donor). Flowers were formed by the receptors regardless of whether or not the donor was photoinduced and capable of floral initiation. We have to assume the existence of two different transmissible stimuli: florigen, the production of which in long- and short-day plants is controlled by daylength, and another stimulus, the production of which in biennials is controlled by low temperature. To stress their separate natures, Melchers (150) has called the temperature-controlled stimulus "vernalin."

If this situation is general, a summer annual long-day plant maintained on short days should induce floral initiation in a noncold-treated biennial just as readily as the long-day-treated short-day plant did. In long-day plants, however, florigen formation is actively inhibited under short-day conditions, and this inhibition somehow acts at a distance. Thus, the situation is not so simple as it appears, and grafts between annual and biennial *Hyoscyamus* gave at first negative results (153). Some recent experiments, however, suggest that by appropriate timing the two effects can to some extent be separated. If the short-day-treated donor was removed 12 days after grafting, flowers were formed by 50 per cent of the receptors (151). The presence of vernalin can be demonstrated in this way in long-day summer annuals, too.

Two basic types of relationship between the two stimuli can then be visualized: (a) vernalin is a precursor of florigen, entering into its formation; and (b) vernalin acts as a catalyst of florigen formation. Attempts to extract vernalin (153) or to obtain a transmission without tissue union (155) have met with no more success than in the case of florigen. We have, therefore, only indirect means to attempt a decision between the two alternatives, but the evidence available is not sufficient to justify a definitive conclusion.

Unlike that of daylength, the effect of low temperature upon floral initiation is of an unmistakably quantitative nature: the longer the treatment, the faster the response, until the optimum level is reached (55, 120, 189, 190). We may safely state (cf. p. 289) that the primary product of vernalization is related to floral initiation in an indirect manner. One may, for example, think of this product as an enzyme catalyzing the synthesis of some compound necessary for floral initiation: the longer the low temperature acts, the more enzyme is formed, and the sooner will the threshold amount of the compound be attained.

At present, however, we do not have any conclusive evidence showing that vernalin is, in fact, the primary product of vernalization. It is just as probable that it is a secondary product catalyzed by some original, cold-induced state localized in the cells. Since vernalin is transmissible, this possibility may even appear as the more plausible one.

On the other hand, even if we were certain that vernalin is a secondary

product of vernalization, we should not be entitled to state definitively that it is a direct florigen precursor. In nonvernalized and in incompletely vernalized winter cereals, flower formation is frequently accelerated by limited periods of short-days ["short-day vernalization" (7, 147, 185, 190, 222)]. If fully vernalized, these plants usually behave like straightforward long-day plants, although in one case flower formation is reported to have been promoted in fully vernalized winter wheat (*Triticum aestivum*) by a period of continuous darkness (235). It appears that the conditions which are optimal for florigen formation are not optimal for the functioning of vernalin. It is possible that vernalin is light-sensitive, but only to such a degree that the sensitivity is not apparent unless the rate of vernalin formation or the amount of vernalin present is low. The existence of short-day vernalization does not rule out the possibility that vernalin is a precursor of florigen, but it does indicate that further processes may intervene. Before this possibility is settled definitive conclusions are premature. It also remains to be seen whether short-day vernalization proves a further clue for the understanding of short-day plants or whether we are dealing with an entirely separate phenomenon.

Photoperiodism and vernalization in retrospect.—The analysis of photoperiodism and vernalization enables us to understand fairly well the general mechanism of the action of daylength and low temperature in relation to floral initiation. What significance do these results have for our understanding of floral initiation in general? The crucial point, as the grafting experiments show, is that flower formation can be induced in a long-day plant and in a short-day plant not only by another long- or short-day plant, but also by a day-neutral plant in which floral initiation is independent of daylength (cf. Table I, p. 270). Likewise, the floral stimulus which in biennial plants appears after low-temperature treatment is also present in summer annuals. This has already been referred to and is substantiated by the summary of grafts given in Table III. In both cases the stimuli pass with equal ease between plants of the same species, between different species, and between different genera. Transmission from host to parasite also seems to be possible (56, 100). The limiting factor thus is not taxonomic relationship but apparently the compatibility of the tissues of donor and receptor.⁹ We thus find that the stimuli demonstrated in plants dependent on daylength and in plants dependent on low temperature are not specific for these plants, but are operative and are identical in all plants. Daylength and temperature de-

⁹ It has been suggested (196) that the transmission of the flowering stimulus in grafts is limited to plants with a "systemic" habit of flowering and does not occur in plants with terminal inflorescences. Transmission, however, has been obtained in the following plants with definitely terminal inflorescences: *Nicotiana*, *Hyoscyamus*, *Brassica*, *Raphanus*, *Daucus*. Thus, the suggestion appears to be no longer valid. The essential conditions—apart from an appropriate treatment of donor and receptor (cf. 121)—seem to be that the tissue union is not limited to xylem elements but also involves the phloem (or perhaps the cortex).

pendence are only special cases in which the formation of the stimuli can be disrupted at specific points by the effect of specific environmental conditions. The significance of the study of the two phenomena lies in the fact that they are footholds for penetrating into the general physiology of floral initiation.

TABLE III

LIST OF FLOWER-INDUCTION GRAFTS BETWEEN NONVERNALIZED COLD-REQUIRING PLANTS AND NONCOLD-REQUIRING OR VERNALIZED COLD-REQUIRING PLANTS

Receptor	Donor (species)	Donor (Response type)*	Refer- ence
<i>Beta vulgaris</i> (sugar beet), biennial strain	<i>Beta vulgaris</i> , biennial strain, cold-treated	B L	4
<i>Beta vulgaris</i> , biennial	<i>Beta vulgaris</i> , annual strain	A L	211
<i>Hyoscyamus niger</i> f. <i>biennis</i>	<i>Hyoscyamus niger</i> f. <i>biennis</i> , cold-treated	B L	149
<i>Hyoscyamus niger</i> f. <i>biennis</i>	<i>Hyoscyamus niger</i> f. <i>annuus</i>	A L	149
<i>Hyoscyamus niger</i> f. <i>biennis</i>	<i>Hyoscyamus albus</i>	A N	149
<i>Hyoscyamus niger</i> f. <i>biennis</i>	<i>Nicotiana tabacum</i> var. Java, Cavalla	A N	149
<i>Hyoscyamus niger</i> f. <i>biennis</i>	<i>Nicotiana tabacum</i> var. Maryland Mammoth	A S	150
<i>Hyoscyamus niger</i> f. <i>biennis</i>	<i>Petunia hybrida</i>	A L?	149
<i>Brassica oleracea</i> (cabbage)	<i>Sinapis spec.</i>	W? L?	4
<i>Sinapis spec.</i>	<i>Brassica oleracea</i>	B N?	4
<i>Daucus carota</i> (carrot)	<i>Anethum graveolens</i> (dill)	A L	4

* Abbreviations:

B biennial plant	L long-day plant
W winter annual plant	S short-day plant
A summer annual plant	N day-neutral plant

An attempt has been made in this review to present the available evidence in a maximally integrated form, but without engaging in speculation unless it helps to clarify the issue. Several authors have advanced hypotheses which are intended to explain in a more specific manner either the entire sequence of the processes underlying floral initiation or certain phases of this sequence (7, 20, 25, 73, 79, 80, 123, 190, 208). Some of these hypotheses are, essentially, generalized transliterations of the experimental findings, while others involve rather detailed assumptions about the chemical or physiological changes leading to floral initiation and about their interrelations. The value of such hypotheses depends largely on the extent of our factual information. If we try to summarize this information, we will have the balance sheet which follows.

We have recognized several individual processes participating in floral

initiation—those involved in its daylength and low-temperature dependence. We cannot be sure, however, that we know all the processes involved in these phenomena and still less that they are all the processes which take part in floral initiation. We know that in either case the processes control the formation of transmissible stimuli. The stimulus appearing as the outcome of the daylength-dependent complex of processes in long-day plants is identical with that in short-day plants. It is the direct outcome of the activity of these processes and controls floral initiation in a direct manner. However, we cannot yet integrate the processes which control the production of this stimulus into one comprehensive story. The stimulus appearing as the outcome of the low-temperature dependent complex of processes is different from and has to be present before the former stimulus can be produced; but we do not know if this stimulus is the direct outcome of the low-temperature dependent processes or the exact relation of the two stimuli. The stimuli are most likely specific chemical compounds, but their exact nature is quite unknown. We have recognized the relation of certain chemical changes to photo- and thermoinduction, thus providing us with the first chemical approaches to flower formation; but this line of work is at its very beginning. We must finally bear in mind that, whereas the general features of photoperiodism and vernalization are well established and have been found to be identical in numerous plants, the physiological work on the two phenomena has been done in many cases with but one or two representatives of each type; its basis, therefore, is very narrow.

This balance shows clearly that there are many broad gaps in our knowledge. Consequently, a hypothesis designed to visualize the relations more clearly and to help in finding new approaches, is perfectly legitimate. If a specific picture is presented, however, it must be borne in mind that it is based not so much on the presence of positive proof as on the lack of sufficient information; it will therefore usually be one of several possibilities among which no decision can yet be made. Such hypotheses, therefore, easily run the risk of offering pseudosolutions of a problem which can be solved only by further experimentation.

SELF-PERPETUATING EFFECTS IN FLORAL INITIATION

In concluding the treatment of photoperiodism and vernalization, a feature must be discussed which may well prove to be one of the most interesting in the entire field. It is presently believed that the appearance of specific, autocatalytic materials is a crucial factor in development, accounting for the stability of the characteristics of differentiated cells and tissues. In floral initiation, also, the existence of such materials appears to be possible. If this possibility is borne out, the situation would be particularly promising, for the formation of the self-perpetuating material would be under the control of definite environmental conditions and thus be accessible to experimental variation.

Indications of self-perpetuating effects are present both in daylength and low-temperature action. In *Xanthium*, the floral stimulus is not only transmitted to noninduced parts of the plant, but these parts continue to produce flowers indefinitely, even if the induced plant part is removed (81). This ability has been passed without any noticeable decrease through several graft "generations" (9; see 11). It is thus difficult to conceive that this ability is caused by the carry-over of a limited, although perhaps ample, amount of the original stimulus. The indirectly induced parts apparently continue to produce the stimulus themselves.

The evidence for self-perpetuating effects in vernalization is more indirect, but even more suggestive. The cold treatment of winter cereals can be applied at amazingly early stages in the life of the plant. It is fully effective not only in seeds in which germination has just begun and then further growth prevented by reduction of the water supply (144), but likewise in seeds developing on the mother plant or on cut-off ears (52, 53, 74, 111, 112). The changes which are caused by thermal induction thus persist through a considerable length of time in which the plant increases enormously in mass. If the treatment is given during embryonic growth, it is more effective in the younger than in the later stages (1). Thus, no loss whatsoever occurs in the subsequent development. Furthermore, if a plant is vernalized in those early stages, the induced state is not limited to the main shoot, but is present to the same degree in the side shoots (tillers), even though they are differentiated long after the end of the treatment. The low-temperature-induced changes seem to be perpetuated as the plant proceeds with its growth and to be maintained in all its cells.

The situation is not so clear in other cases. Photoperiodic induction can persist for extended periods of time, but in most plants it is definitely not permanent, provided the inductive treatment was not so extended as to determine all growing points of the plant to floral initiation (see 44, 45, 121, 143). According to Chouard (45), some LDP (*Helianthemum guttatum*, *Nigella damascena*, etc.), once photoinduced, do not revert to vegetative growth; but other cases comparable to that of *Xanthium* in which there is a maintenance of the induction through several graft generations have so far not been reported. In biennial plants, cold treatment of germinating seed is either not effective (cf. 151), or the effect is comparatively slight (175, 227). Treatment during seed development has not been reported. In *Hyoscyamus* the effect of optimal thermal induction does not noticeably decrease in the course of at least 100 days (124), but it has been observed that the effect of a suboptimal induction disappears in a shorter period (121). However, even in these cases the presence of a self-perpetuating material is not ruled out, for its rate of reproduction may lag behind the growth rate of the plant so that a continuous refilling is required.

We are thus led to consider the possibility that the effects of both photo- and thermoinduction are self-perpetuating. Purvis (189) believes that the

course of vernalization itself—that is, the increase of the effect during the actual cold treatment—is autocatalytic. The multiplication of the self-perpetuating materials would be closely co-ordinated with the growth of the plant, for both in *Xanthium* and in winter cereals, differences in the degree of induction persist throughout the life of the plant as differences in the intensity and regularity of flowering or in the earliness of the response. We would seem to be dealing with materials which multiply regularly along with cell division. The multiplication would seem mainly to occur in the meristematic tissues, for in *Xanthium* only those noninduced parts of the plant in a meristematic condition at the time of the initial photoinduction are capable of the indirect induction (134). In the case of *Xanthium* we could assume that the self-perpetuating effect is identical with the floral stimulus which appears as the result of photoinduction. This would indicate that florigen is autocatalytic and would be another reason why it cannot be detached from living cells. In vernalization, the autocatalytic material may not be identical with the transmissible stimulus, but may be strictly intracellular, for we cannot decide whether vernalin is a direct or a secondary product of cold action (see above, p. 293).

THE LATER STAGES OF FLOWERING

The later stages of flower development.—Our insight into the physiology of the later stages of flowering is very deficient and consists largely of isolated pieces of evidence. The separation of some of the stages may seem questionable, particularly that between floral initiation and organization and that between floral maturation and anthesis. In most cases, a floral primordium, once initiated, continues to develop at least into a complete, microscopical bud, and once a flower has matured, it enters anthesis *quasi* automatically. In a few instances, however, this close relationship is broken, indicating that the stages do differ in some specific way. We have already seen that in *Xanthium*, floral initiation involves a reduction of the auxin level in the dark periods of photoinduction, whereas the further development of the inflorescence primordia is dependent on a sufficient auxin supply (p. 279). Similarly, in certain plants the unfolding of the flowers depends on a definite environmental stimulus and withholding this stimulus results in a delay of anthesis [for example, in *Cereus grandiflorus* (199)].

The stimuli of floral initiation and the later stages of flowering.—Since floral initiation is determined by specific stimuli, an obvious question is whether or not these stimuli also have some part in the further development of the flower. Most evidence indicates that they have. The time of floral initiation does not depend on the amount of photoinduction once the threshold value has been reached (p. 289); but both the number of flowers initiated and the rate and degree of their development usually increase as the inductive treatment is extended (for example, 79, 95, 121). In vernalization, not only the time to initiation is shortened as the duration of thermal in-

duction is increased, but also the quantity of flower formation [for example, the number of spikelets formed (7)] seems to increase. Some observations, however, indicate that the floral-initiating stimulus is not the only factor which controls the subsequent development of the flowers. The development of the differentiated floral primordia towards anthesis depends in some plants on environmental conditions different from those which determine initiation (60). *Hyoscyamus* plants which have received a suboptimal thermal- or photoinduction produce great numbers of floral primordia, which, however, develop only to small defective buds (121). In *Fragaria*, a substance has been extracted from photoinduced plants which promotes maturation of the initiated buds (205).

Reduction of the intensity of flowering is frequently accompanied by leaf-like development (phyllody) of the leaf organs (bracts) in the flower region. In *Kalanchoë*, however, the two phenomena have different causative mechanisms: the number of flowers depends on the amount of the floral stimulus, but the phyllody depends on the shortness of the dark periods which the plants receive either during or after photoinduction (89).

Flower development and auxin.—The growth of the flowers proceeds at first by cell division; later, cell elongation becomes a major factor, except in the ovary which may continue to grow to maturity almost exclusively by cell division (cf. 101, 202, 203, 207, 219, 220, 221). A priori, it is to be expected that auxin plays an essential part, at least in floral maturation. It seems, furthermore, that auxin is already essential in the division period of growth, for the ovaries of *Cucumis anguria* (gherkin) contain auxin from the very earliest stages of growth, and the decrease in growth rate prior to full maturity (before fertilization) coincides with a drop in the auxin content (172). It appears likely that the separation of the division and the elongation periods of growth in organs with a limited growth capacity is in general not warranted. The transition of the growth of Cucurbit ovaries from division to elongation—which occurs, moreover, in different tissues at different times—is not at all reflected in the growth rate of the whole ovary (204). It seems that cell division in such organs is an essentially passive process, determined by the size increase of the cells and the supply of materials to the fruit. The behavior of *Xanthium* shows that in the youngest stages the flowers or inflorescences are dependent on an auxin supply from other parts of the plant. In later stages, they produce auxin themselves, and this auxin may also control the growth of the flower or inflorescence stalks (6, 7, 97, 209). The time of onset of auxin production apparently varies in different species. In *Secale* it seems to be as late as the time of ear emergence (97). Most of the auxin is formed in the anthers (97, 234), where it undergoes a definite developmental cycle (97). If the anthers are removed, the growth of the entire flower bud (236) or of certain of its parts, like the pistil and the hypanthium of *Oenothera* flowers (226), may cease. The auxin of the anthers seems to serve as an auxin source for the other parts of the flower, with the exception of the ovary

which produces its own auxin (see above). Removal of the anthers is effective only prior to a certain point of development; later, the flower parts have apparently received enough auxin to finish development.

Differential effects in the development of male and female flower parts and flowers.—In general, the development of the different parts of a flower is highly co-ordinated and if conditions are unfavorable all parts cease growing simultaneously. In certain cases, however, a differential response of anthers and ovaries has been observed. In *Lycopersicum esculentum* (tomato) (102) and in *Bryophyllum* (194) low light intensity causes failure of anther development, while in *Capsella bursa-pastoris* the same effect is caused by unfavorable temperature and water supply conditions (115). Particularly interesting are those cases in which the sex differentiation in monoecious plants is affected. In *Xanthium* (171) and in *Ambrosia* (146) the ratio of female and male inflorescences or flowers is increased with extended short-day treatment; the total number may remain the same. In *Cucurbita pepo* (Acorn squash), low temperatures and short photoperiods favor the production of female over male flowers (173). Such cases may prove a tool for studying floral organization, a stage which has so far defied any experimental approach. It is possible that the effect of the environmental conditions is exerted through changes in the auxin level. Some of the effective conditions (short days, low temperatures) are believed to reduce the auxin level (although the evidence is hardly conclusive). In *Cucumis sativus* (cucumber) and in *Cucurbita*, auxin application causes formation of female flowers in sites which are normally occupied by male ones (116, 173).

LITERATURE CITED

1. Aginān, A. A., *Agrobiologiya*, No. 3, 57 (1950)
2. Allard, H. A., *J. Agr. Research*, **57**, 775 (1938)
3. Allard, H. A., and Garner, W. W., *J. Agr. Research*, **63**, 305 (1941)
4. Avakīan, A. A., *Agrobiologiya*, No. 2, 12 (1950)
5. Avakīan, A. A., and ĭastreb, M. G., *Agrobiologiya*, No. 6, 41 (1949)
6. Bakhuyzen, H. L. van de S., *Landbouwkund. Tijdschr.*, **55**, 533 (1943)
7. Bakhuyzen, H. L. van de S., *Verslag. Landbouwk. Onderzoek.*, [B]**53**, 145 (1947)
8. Bonner, J., *Botan. Gaz.*, **110**, 625 (1949)
9. Bonner, J. (Unpublished data)
10. Bonner, J., and Bonner, D., *Botan. Gaz.*, **110**, 154 (1948)
11. Bonner, J., and Liverman, J. L., *Growth* (In press)
12. Bonner, J., and Liverman, J. L. (Unpublished data)
13. Bonner, J., and Thurlow, J., *Botan. Gaz.*, **110**, 613 (1949)
14. Borthwick, H. A. (Unpublished data)
15. Borthwick, H. A., Hendricks, S. B., and Parker, M. W., *Botan. Gaz.*, **110**, 103 (1948)
16. Borthwick, H. A., Hendricks, S. B., and Parker, M. W., *Botan. Gaz.*, **113**, 95 (1951)
17. Borthwick, H. A., and Parker, M. W., *Botan. Gaz.*, **100**, 374 (1938)
18. Borthwick, H. A., and Parker, M. W., *Botan. Gaz.*, **101**, 806 (1940)
19. Borthwick, H. A., Parker, M. W., and Heinze, P. H., *Botan. Gaz.*, **102**, 792 (1941)
20. Borthwick, H. A., Parker, M. W., and Hendricks, S. B., in *Vernalization and Photoperiodism*, 71 (Murneek, A. E., and Whyte, R. O., Eds., *Chronica Botanica*, Waltham, Mass., 196 pp., 1948)
21. Borthwick, H. A., Parker, M. W., and Hendricks, S. B., *Am. Naturalist*, **84**, 117 (1950)
22. Bünning, E., *Ber. deut. botan. Ges.*, **54**, 590 (1937)
23. Bünning, E., *Biol. Zentr.*, **64**, 161 (1944)
24. Bünning, E., *Flora*, **38**, 93 (1944)
25. Bünning, E., *Naturwissenschaften*, **33**, 271 (1946)
26. Bünning, E., *Z. Naturforsch.*, **3b**, 457 (1948)
27. Bünning, E., *Planta*, **38**, 521 (1950)
28. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **12**, 443 (1936)
29. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **13**, 79 (1936)
30. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **16**, 227 (1937)
31. Ćallahfān, M. H., *Hormone Theory of Plant Development* (Moscow-Leningrad, Acad. of Sci. USSR, 198 pp., 1937)
32. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **18**, 607 (1938)
33. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **27**, 160 (1940)
34. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **27**, 253 (1940)
35. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **27**, 370 (1940)
36. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **31**, 949 (1941)
37. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **47**, 220 (1945)
38. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **54**, 735 (1946)
39. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **54**, 837 (1946)
40. Ćallahfān, M. H., *Compt. rend. acad. sci. U.R.S.S.*, **55**, 69 (1947)
41. Ćallahfān, M. H., and ĭarkovaĭ, L. M., *Compt. rend. acad. sci. U.R.S.S.*, **15**, 215 (1937)

42. Čallahfan, M. H., and Samygin, G. A., *Doklady Akad. Nauk S.S.S.R.*, **56**, 187 (1948)
43. Chouard, P., *Compt. rend.*, **219**, 469 (1944)
44. Chouard, P., *Bull. soc. botan. France*, **96**, 106 (1949)
45. Chouard, P., *Compt. rend.*, **231**, 1245 (1950)
46. Chouard, P., and Poignant, P., *Compt. rend.*, **232**, 103 (1951)
47. Chroboczek, E., *Cornell Univ. Agr. Expt. Sta. Mem.*, No. 154 (1934)
48. Claes, H., *Z. Naturforsch.*, **2b**, 45 (1947)
49. Claes, H., *Z. Naturforsch.*, **7b**, 50 (1952)
50. Claes, H., and Lang, A., *Z. Naturforsch.*, **2b**, 56 (1947)
51. Correns, C. E., *Ber. deut. botan. Ges.*, **22**, 517 (1904)
52. Crescini, F., *Z. Zücht.*, [A]**20**, 492 (1935)
53. Crescini, F., *Z. Zücht.*, [A]**21**, 201 (1936)
54. Curtis, O. F., and Chang, H. T., *Am. J. Botany*, **17**, 1047 (1930)
55. von Denffer, D., *Jahrb. wiss. Botan.*, **88**, 759 (1939)
56. von Denffer, D., *Biol. Zentr.*, **67**, 175 (1948)
57. von Denffer, D., *Naturwissenschaften*, **37**, 296 and 317 (1950)
58. von Denffer, D., and Gründler, H., *Biol. Zentr.*, **69**, 272 (1950)
59. von Denffer, D., and Schlitt, L., *Naturwissenschaften*, **38**, 564 (1951)
60. Eguchi, T., *Proc. Imp. Acad. (Tokyo)*, **13**, 332 (1937)
61. Efeikin, A. K., *Compt. rend. acad. sci. U.R.S.S.*, **25**, 308 (1939)
62. Efeikin, A. K., *Compt. rend. acad. sci. U.R.S.S.*, **30**, 656 (1941)
63. Eremenko, V. T., *Sovet. Botan.*, No. 6, 36 (1935)
64. Fabian, I., *Z. Botan.*, **33**, 305 (1938)
65. Filippenko, I. A., *Compt. rend. acad. sci. U.R.S.S.*, **28**, 167 (1940)
66. Funke, G. L., *Natuurw. Tijdschr.*, (Belg.), **15**, 209 (1933)
67. Funke, G. L., *Biol. Jaarb.*, **4**, 345 (1937)
68. Funke, G. L., in *Vernalization and Photoperiodism*, 79 (Murneek, A. E., and Whyte, R. O., Eds., *Chronica Botanica*, Waltham, Mass., 196 pp. 1948)
69. Galston, A. W., *Botan. Gaz.*, **110**, 495 (1949)
70. Gentscheff, G., and Gustafsson, Å., *Hereditas*, **26**, 250 (1940)
71. Gerhard, E., *J. Landw.*, **87**, 161 (1940)
72. Goebel, C., *Die Physiologie der Vernalisationsvorgänge* (College Teacher's Degree Thesis, Univ. of Tübingen, 1950)
73. Gregory, F. G., *Symposia Soc. Exptl. Biol.*, **2**, 75 (1948)
74. Gregory, F. G., and Purvis, O. N., *Ann. Botany*, **2**, 237 (1938)
75. Gregory, F. G., and Purvis, O. N., *Ann. Botany*, **2**, 753 (1938)
76. Gregory, F. G., and Purvis, O. N., *Nature*, **161**, 859 (1948)
77. Greulach, V. A., *Botan. Gaz.*, **103**, 698 (1942)
78. Greulach, V. A., *Ohio J. Sci.*, **43**, 65 (1943)
79. Hamner, K. C., *Botan. Gaz.*, **101**, 658 (1940)
80. Hamner, K. C., *Symposia Soc. Exptl. Biol.*, **2**, 104 (1948)
81. Hamner, K. C., and Bonner, J., *Botan. Gaz.*, **100**, 388 (1938)
82. Hamner, K. C., and Naylor, A. W., *Botan. Gaz.*, **100**, 853 (1939)
83. Harder, R., *Flora*, **38**, 1 (1944)
84. Harder, R., and Bode, O., *Planta*, **33**, 469 (1943)
85. Harder, R., Bode, O., and von Witsch, H., *Flora*, **36**, 85 (1942)
86. Harder, R., Bode, O., and von Witsch, H., *Jahrb. wiss. Botan.*, **91**, 381 (1944)
87. Harder, R., and Gall, E., *Nachr. Ges. Wiss. Göttingen, Math.-physik. Klasse*, **54** (1945)

88. Harder, R., and Gümmer, G., *Planta*, **35**, 88 (1947)
89. Harder, R., and Gümmer, G., *Biol. Zentr.*, **68**, 435 (1949)
90. Harder, R., and van Senden, H., *Naturwissenschaften*, **36**, 348 (1949)
91. Harder, R., and Springorum, B., *Biol. Zentr.*, **66**, 147 (1947)
92. Harder, R., Wallrabe, E., and Quantz, L., *Planta*, **34**, 41 (1944)
93. Harder, R., Westphal, M., and Behrens, G., *Planta*, **36**, 424 (1948)
94. Harder, R., and von Witsch, H., *Gartenbauwiss.*, **15**, 226 (1940)
95. Harder, R., von Witsch, H., and Bode, O., *Jahrb. wiss. Botan.*, **90**, 546 (1942)
96. Hartmann, H. T., *Plant Physiol.*, **22**, 407 (1947)
97. Hatcher, E. S. J., *Ann. Botany*, **9**, 235 (1945)
98. Hauschild, I., *Über die an der photoperiodischen Reaktion der Kurztagpflanzen beteiligten Vorgänge* (Doctoral thesis, Univ. of Göttingen, 1943)
99. Heinze, P. H., Parker, M. W., and Borthwick, H. A., *Botan. Gaz.*, **103**, 517 (1942)
100. Holdsworth, H., and Nutman, P. S., *Nature*, **160**, 223 (1947)
101. Houghtaling, H. B., *Bull. Torrey Botan. Club*, **62**, 243 (1935)
102. Howlett, F. S., *J. Agr. Research*, **58**, 79 (1939)
103. Jerchel, D., and Müller, R., *Naturwissenschaften*, **38**, 561 (1951)
104. Katunskii, V. N., *Compt. rend. acad. sci. U.R.S.S.*, **15**, 507 (1937)
105. Khudairi, A. (Unpublished data)
106. Klein, W. H., and Leopold, A. C. (Unpublished data)
107. Klešnin, A. F., *Compt. rend. acad. sci. U.R.S.S.*, **52**, 813 (1946)
108. Knott, J., *Proc. Am. Soc. Hort. Sci.*, **31**, Suppl., 152 (1934)
109. Knott, J., *Cornell Univ. Agr. Expt. Sta. Mem.*, No. 218 (1939)
110. Kononov, I. N., *Compt. rend. acad. sci. U.R.S.S.*, **16**, 381 (1936)
111. Kostŭčenko, I. A., and Zarubaŭlo, T. F., *Selekcija i Semenovodstvo*, No. 3, 49 (1935)
112. Kostŭčenko, I. A., and Zarubaŭlo, T. F., *Zhur. Inst. Botan. Akad. Nauk U.R.-S.R.*, **18/19**, 81 (1938)
113. Kuijper, J., and Wiersum, L. K., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **39**, 1114 (1936)
114. Laibach, F., *Beitr. Biol. Pflanz.*, **28**, 173 (1951)
115. Laibach, F., *Ber. deut. botan. Ges.*, **64**, 135 (1951)
116. Laibach, F., and Kribben, F. J., *Ber. deut. botan. Ges.*, **63**, 119 (1950)
117. Lang, A., *Biol. Zentr.*, **61**, 427 (1941)
118. Lang, A., *Z. ind. Abstamm. Vererbungsl.*, **80**, 210 (1942)
119. Lang, A., *Z. Naturforsch.*, **2b**, 36 (1947)
120. Lang, A., *Züchter*, **21**, 241 (1951)
121. Lang, A. (Unpublished data)
122. Lang, A., and Melchers, G., *Naturwissenschaften*, **29**, 82 (1941)
123. Lang, A., and Melchers, G., *Planta*, **33**, 653 (1943)
124. Lang, A., and Melchers, G., *Z. Naturforsch.*, **2b**, 444 (1947)
125. Lang, A., and Melchers, G., *Z. Naturforsch.*, **3b**, 108 (1948)
126. Leopold, A. C. (Unpublished data)
127. Leopold, A. C., and Thimann, K. V., *Am. J. Botany*, **36**, 342 (1949)
128. Lincoln, R. G., and Raven, K. A. (Unpublished data)
129. Linser, H., *Planta*, **39**, 377 (1951)
130. Lübbimenko, V. N., and Buslova, E. D., *Compt. rend. acad. sci. U.R.S.S.*, **14**, 149 (1937)
131. Liverman, J. L., *The Physiology and Biochemistry of Flowering* (Doctoral thesis, California Institute of Technology, 1952)

132. Liverman, J. L., and Lang, A. (Unpublished data)
133. Loehwing, W. F., *Proc. Soc. Exptl. Biol. Med.*, **37**, 631 (1938)
134. Lona, F., *Nuovo giorn. botan. ital.*, **53**, 548 (1946)
135. Lona, F., *Nuovo giorn. botan. ital.*, **53**, 635 (1946)
136. Lona, F., in *Lavori di Botanica*, 277 (G. Gola Jubilee Vol., Padova, Italy, 1947)
137. Lona, F., in *Lavori di Botanica*, 285 (G. Gola Jubilee Vol., Padova, Italy, 1947)
138. Lona, F., *Nuovo giorn. botan. ital.*, **55**, 559 (1948)
139. Lona, F., *Nuovo giorn. botan. ital.*, **56**, 479 (1949)
140. Lona, F., *Boll. soc. ital. biol. sper.*, **25**, 761 (1949)
141. Lona, F., *Rend. ist. lombardo sci.*, (III), **83** (14), 1 (1950)
142. Lona, F., *Humus (Milan)*, **6** (4), 6 (1950)
143. Long, E. M., *Botan. Gaz.*, **101**, 168 (1939)
144. Lysenko, T. D., *Trudy Azerbaizhdzhan. tsentr. Opyt. Selekc. Stanc.*, No. 3, 168 pp. (1928)
145. Mann, L. K., *Botan. Gaz.*, **102**, 339 (1940)
146. Mann, L. K., *Botan. Gaz.*, **103**, 780 (1942)
147. McKinney, H. H., and Sando, W. J., *J. Agr. Research*, **51**, 621 (1935)
148. Melchers, G., *Biol. Zentr.*, **56**, 567 (1936)
149. Melchers, G., *Biol. Zentr.*, **57**, 568 (1937)
150. Melchers, G., *Ber. deut. botan. Ges.*, **57**, 29 (1939)
151. Melchers, G. (Unpublished data)
152. Melchers, G., and Claes, H., *Naturwissenschaften*, **31**, 249 (1943)
153. Melchers, G., and Lang, A., *Biol. Zentr.*, **61**, 16 (1941)
154. Melchers, G., and Lang, A., *Naturwissenschaften*, **30**, 589 (1942)
155. Melchers, G., and Lang, A., *Z. Naturforsch.*, **3b**, 105 (1948)
156. Melchers, G., and Lang, A., *Biol. Zentr.*, **67**, 105 (1948)
157. Melchers, G., and Lang, A. (Unpublished data)
158. Michener, H. D., *Am. J. Botan.*, **29**, 558 (1942)
159. Moškov, B. S., *Trudy Priklad. Botan. Genetike i Selektivii*, [A]17, 25 (1936)
160. Moškov, B. S., *Trudy Priklad. Botan. Genetike i Selektivii*, [A]19, 107 (1936)
161. Moškov, B. S., *Trudy Priklad. Botan. Genetike i Selektivii*, [A]21, 145 (1937)
162. Moškov, B. S., *Compt. rend. acad. sci. U.R.S.S.*, **22**, 460 (1939)
163. Moškov, B. S., *Compt. rend. acad. sci. U.R.S.S.*, **24**, 489 (1939)
164. Moškov, B. S., *Sovet. Botan.*, No. 4, 32 (1940)
165. Moškov, B. S., *Compt. rend. acad. sci. U.R.S.S.*, **31**, 161 (1941)
166. Moškov, B. S., *Doklady Akad. Nauk S.S.S.R.*, **71**, 391 (1950)
167. Murneek, A. E., *Botan. Gaz.*, **102**, 269 (1940)
168. Murneek, A. E., and Whyte, R. O., Eds., *Vernalization and Photoperiodism* (Chronica Botanica, Waltham, Massachusetts, 196 pp. 1948)
169. Naylor, A. W., *Botan. Gaz.*, **102**, 557 (1941)
170. Naylor, A. W., *Botan. Gaz.*, **103**, 342 (1941)
171. Naylor, F. L., *Botan. Gaz.*, **103**, 146 (1941)
172. Nitsch, J. P., *The Role of Plant Hormones in Fruit Development* (Doctoral thesis, California Institute of Technology, 1950)
173. Nitsch, J. P., Kurtz, E. B., Jr., Liverman, J. L., and Went, F. W., *Am. J. Botany*, **39**, 32 (1952)
174. Olefnikova, T. V., *Doklady Akad. Nauk S.S.S.R.*, **68**, 605 (1949)
175. Owen, F. V., Carsner, E., and Stout, M., *J. Agr. Research*, **61**, 101 (1940)
176. Parker, M. W., and Borthwick, H. A., *Botan. Gaz.*, **101**, 145 (1939)
177. Parker, M. W., and Borthwick, H. A., *Botan. Gaz.*, **102**, 256 (1940)

178. Parker, M. W., and Borthwick, H. A., *Botan. Gaz.*, **104**, 612 (1943)
179. Parker, M. W., and Borthwick, H. A., *Ann. Rev. Plant Physiol.*, **1**, 43 (1950)
180. Parker, M. W., Hendricks, S. B., and Borthwick, H. A., *Botan. Gaz.*, **111**, 242 (1950)
181. Parker, M. W., Hendricks, S. B., Borthwick, H. A., and Scully, N. J., *Botan. Gaz.*, **108**, 1 (1946)
182. Parker, M. W., Hendricks, S. B., Borthwick, H. A., and Went, F. W., *Am. J. Botany*, **36**, 194 (1949)
183. Parker, M. W., Wildman, S. G., and Campbell, J. M. (Unpublished data)
184. Psarev, G. M., *Sovet. Botan.*, No. 3, 88 (1936)
185. Purvis, O. N., *Ann. Botany*, **48**, 919 (1934)
186. Purvis, O. N., *Nature*, **145**, 462 (1940)
187. Purvis, O. N., *Ann. Botany*, **7**, 285 (1944)
188. Purvis, O. N., *Ann. Botany*, **11**, 270 (1947)
189. Purvis, O. N., *Ann. Botany*, **12**, 183 (1948)
190. Purvis, O. N., and Gregory, F. G., *Ann. Botany*, **1**, 569 (1937)
191. Purvis, O. N., and Gregory, F. G., *Nature*, **155**, 113 (1945)
192. Razumov, V. J., in *K. A. Timirâzev Memorial Volume of Plant Physiology Research*, 283 (Academy of Sciences U.R.S.S., Moscow, 1941)
193. Resende, F., *Bol. soc. portug. cienc. nat.*, [2a]2, 174 (1949)
194. Resende, F., *Portugaliae Acta Biol.*, [A]2, 365 (1949)
195. Roberts, R. H., in *Plant Growth Substances*, 347 (Skoog, F., Ed., Univ. Wisconsin Press, Madison, Wis., 476 pp., 1951)
196. Roberts, R. H., and Struckmeyer, B. E., *Science*, **90**, 16 (1939)
197. Roberts, R. H., and Struckmeyer, B. E., *J. Agr. Research*, **59**, 699 (1939)
198. Schmitz, J., *Planta*, **39**, 271 (1951)
199. Schmucker, T., *Planta*, **5**, 549 (1938)
200. Sen, B., and Chakravarti, S. C., *Nature*, **159**, 783 (1947)
201. van Senden, H., *Biol. Zentr.*, **70**, 537 (1951)
202. Sinnott, E. W., *Am. J. Botany*, **26**, 179 (1939)
203. Sinnott, E. W., *Am. J. Botany*, **32**, 439 (1945)
204. Sinnott, E. W., *Growth*, **9**, 189 (1945)
205. Sironval, C., *Bull. classe sci. Acad. roy. Belg.*, **36**, 779 (1950)
206. Skvorcov, S. S., *Compt. rend. acad. sci. U.R.S.S.*, **55**, 773 (1947)
207. Smith, W. H., *Ann. Botany*, **14**, 23 (1950)
208. Snyder, W. E., *Am. J. Botany*, **35**, 520 (1948)
209. Söding, H., *Flora*, **32**, 425 (1938)
210. Steinberg, R. A., and Garner, W. W., *J. Agr. Research*, **52**, 943 (1936)
211. Stout, M., *Botan. Gaz.*, **107**, 86 (1945)
212. Stout, M., *J. Agr. Research*, **72**, 49 (1946)
213. Strydom, J. C., *Effects of Ethylene Chlorhydrin on the Rest Period and Auxin Content in Gladiolus Corms*. (Doctoral thesis, Univ. of California in Los Angeles, 1950)
214. Taravet, A., *Compt. rend.*, **224**, 1373 (1947)
215. Taravet, A., *Compt. rend.*, **224**, 1443 (1947)
216. Tetûrev, V. A., *Botan. Zhurn. S.S.S.R.*, **25**, 505 (1940)
217. Thurlow, J., *Certain Aspects of Photoperiodism* (Doctoral thesis, California Institute of Technology, 1948)
218. Thurlow, J., and Bonner, J., *Am. J. Botany*, **34**, 603 (1947)
219. Tukey, H. B., *Proc. Am. Soc. Hort. Sci.*, **30**, 209 (1939)

- 220. Tukey, H. B., and Young, J. O., *Botan. Gaz.*, **100**, 723 (1939)
- 221. Tukey, H. B., and Young, J. O., *Botan. Gaz.*, **104**, 3 (1942)
- 222. Voss, J., *Pflanzenbau*, **15**, 1 and 49 (1938)
- 223. Wallrabe, E., *Botan. Arch.*, **45**, 281 (1944)
- 224. Wassink, E. C., and van der Scheer, C., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **53**, 1064 (1950)
- 225. Wassink, E. C., Sluisman, C. M. J., and Stolwijk, J. A. J., *Proc. Koninkl. Nederland. Akad. Wetenschap.*, **53**, 1466 (1950)
- 226. Weinland, H., *Z. Botan.*, **36**, 401 (1941)
- 227. Wellensiek, S. J., and Verkeerck, K., *Mededeel., Direct. Tuinbouw*, **13**, 341 (1950)
- 228. Withrow, A. P., and Withrow, R. B., *Botan. Gaz.*, **104**, 409 (1943)
- 229. Withrow, A. P., Withrow, R. B., and Biebel, J. P., *Plant Physiol.*, **18**, 294 (1943)
- 230. Withrow, R. B., and Benedict, H. M., *Plant Physiol.*, **11**, 225 (1936)
- 231. Withrow, R. B., and Biebel, J. P., *Plant Physiol.*, **11**, 807 (1936)
- 232. Withrow, R. B., and Withrow, A. P., *Plant Physiol.*, **15**, 609 (1940)
- 233. von Witsch, H., *Planta*, **31**, 638 (1941)
- 234. Wittwer, S. H., *Agric. Exptl. Sta. Mo. Bull.*, No. 371 (1943)
- 235. Wort, D. J., *Botan. Gaz.*, **102**, 725 (1941)
- 236. Zanoni, G., *Ann. Botanica*, **22**, 46 (1941)
- 237. Zdanova, L. P., *Compt. rend. acad. sci. U.R.S.S.*, **49**, 62 (1945)
- 238. Zdanova, L. P., *Doklady Akad. Nauk S.S.S.R.*, **70**, 715 (1950)

CARBOXYLATING ENZYMES IN PLANTS¹

BY BIRGIT VENNESLAND AND ERIC E. CONN

Department of Biochemistry, University of Chicago, Chicago, Illinois

The scope of this review is not limited completely to papers which deal directly with the subject of plant carboxylases but includes some discussion of the functional relationship of carboxylase-catalyzed reactions to the overall process of metabolism in plant tissues. Because of the demonstrated value of the comparative approach, certain aspects of enzyme studies on material other than plants is covered also, insofar as such work may provide analogies helpful to the study of plant metabolism.

Most of the recent working hypotheses regarding the mechanism of carbon dioxide assimilation in photosynthesis contain an assumption that some of the reactions whereby carbon dioxide and water are converted, photosynthetically, into carbohydrate represent a reversal of the oxidative degradation of the carbohydrate. The current interest which attaches to carboxylases of plants is partly a result, therefore, of the possibility that the reactions catalyzed by these enzymes are reversible. This aspect of the problem will consequently be stressed. Since the path of carbon in photosynthesis has been covered at length in previous volumes of this series [Benson & Calvin (1), Gaffron & Fager (2)], discussion of this phase of the subject will be limited to the contributions of the past year. The review of Goddard & Meeuse (3) is likewise taken as the point of departure for the discussion of the respiratory release of carbon dioxide in plants.

THE KREBS CYCLE IN PLANTS

In 1949, Krebs (4) reviewed the criteria which must be satisfied to justify the conclusion that the respiratory breakdown of pyruvate occurs by way of the Krebs or citrate cycle in a particular tissue. Furthermore, he emphasized his view that conclusive evidence for the functional operation of the cycle existed, at that time, only for animal tissues, but not for plants and yeast. In a later discussion (5), he expressed the opinion that the cycle does occur in yeast and some bacteria, but that it is probably not "the major pathway of respiration," being used instead mainly for synthetic purposes (e.g., to form an amino acid precursor like α -ketoglutaric acid).

The question of the occurrence of the Krebs cycle in higher plants should be considered from two points of view. First, in what species and tissues has the cycle been demonstrated to occur? Second, what is the function of the cycle in any particular tissue where it may be demonstrated?

Two years ago, Goddard & Meeuse (3) reviewed the evidence available at the time for the existence of the citrate cycle in higher plants. Most of this

¹ The survey of the literature pertaining to this review was concluded in December, 1951.

evidence was indirect. In the case of spinach leaf and barley root, the experimental support was strong, though not completely conclusive. The greatest technical difficulty which confronted investigators was the necessity of working with intact cells. Complete proof of the cycle should include evidence that each individual component of the reaction sequence is formed from its immediate precursor(s). The existence of permeability barriers and side reactions in intact cells create serious obstacles to the acquisition of such data.

It is noteworthy, therefore, that Millerd *et al.* (6) have succeeded in obtaining, from etiolated mung beans (*Phaseolus aureus*), a cell-free preparation which catalyzes the aerobic oxidation of all the members of the cycle. Pyruvate is completely oxidized to carbon dioxide and water, and the dicarboxylic acids have a catalytic effect on this oxidation. The enzyme preparation consisted of washed particles isolated by centrifugation. A solution of sucrose and phosphate buffer was used as the suspending medium. Proper choice of medium was critical for obtaining active preparations. The absence of chlorophyll ensured lack of interference by the photosynthetic reactions. The particles are strikingly similar in enzyme content and behavior to the washed particulate preparations from animal tissues which have long been known to catalyze the Krebs cycle. Such preparations from animal tissues are frequently termed "mitochondria" and the name has also been applied to the plant preparation.

The plant "mitochondria" accumulate high energy phosphate at the expense of the energy released by the oxidation, and the authors are of the opinion that the cytochrome system is used to activate electron transport to oxygen. In view of the demonstrated similarity between plant and animal "mitochondria," it seems reasonable to conclude that the mung bean seedling contains an organized, insoluble enzyme structure which oxidizes pyruvate by a mechanism similar in all its main features to the Krebs cycle of animal tissues. Further experimental evidence bearing on this point may be expected to accumulate rapidly. Dr. Helen Stafford, in unpublished experiments, has found that particles prepared from pea seedlings (*Pisum sativum*) by the procedure of Millerd *et al.* likewise catalyze the oxidation of pyruvate and the other acids of the cycle. It will be of interest to see to what extent the enzymically active "mitochondria" can be prepared from plant tissues generally. The question of the presence of such particles in the mature green leaf and of the identity or separate existence of chloroplasts and "mitochondria" are particularly pertinent.

One of the main objections to the occurrence of the citrate cycle in plant tissues has been based on the difficulty of demonstrating succinic dehydrogenase activity in cell-free plant preparations. Recent success in the demonstration of this enzyme indicates, however, that it may be found to be widespread, provided adequate preparative and testing procedures are employed [Stafford (7), Price & Thimann (8), Hasse & Fruhstorfer (9)].

Results obtained by several workers in studies of the metabolism of plant acids in intact tissues can be explained in part by the operation of a Krebs

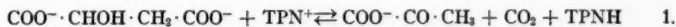
cycle. Thus, stimulating effects of various acids of the cycle on respiration have been observed by Morrison (10) with starved rhubarb leaves. Observations on the properties of aconitase in this plant were also made. Studies of malonate inhibition of leaf respiration gave irregular results. Boswell (11) found that root slices of *Brassica napus* L. metabolize the four carbon dicarboxylic acids as well as pyruvate, citrate, and α -ketoglutarate. Pyruvic carboxylase, and probably oxalacetic carboxylase also, were present. Neither Morrison nor Boswell attempted to draw definite conclusions about the occurrence of the citrate cycle. Barrón *et al.* (12) have examined the respiration of potato slices. Some of the acids of the cycle stimulated respiration, acetate was metabolized, and pyruvic carboxylase was present. In addition, an enzyme which synthesizes citrate from oxalacetate, acetate, and adenosinetriphosphate (ATP) was found. Although α -ketoglutarate did not stimulate respiration, it disappeared during the incubation period. The results were regarded as evidence for the occurrence of the Krebs cycle.

Foulkes (13) has obtained yeast extracts which metabolized tricarboxylic acids at rates of about 40 per cent of the rate of oxidation of pyruvate by intact cells, although the intact cells failed to act on added tricarboxylic acids. Eny (14) has studied the effect of organic acids on the respiration of *Chlorella*. Lactic, pyruvic, acetic, aconitic, citric, α -ketoglutaric, succinic, fumaric, malic, butyric, and propionic acid were tested. All these acids were utilized for respiration and growth. The conclusion was drawn that the path through which the oxidation of carbohydrate is effected by *Chlorella* must have much similarity with the tricarboxylic acid cycle. The results of Stutz & Burris (15), which are discussed further on p. 316, indicate that a Krebs cycle may operate in leaves, but only sluggishly, in comparison with certain other metabolic reactions.

The cumulative evidence of the work cited in this section indicates that all the reactions of the Krebs cycle probably occur in some and perhaps in many plant tissues; but it is too early to assess the precise role of the cycle or its quantitative importance in respiration. Much of the pertinent information on the subject is summarized in a review by Laties (16).

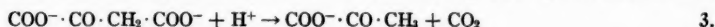
CARBOXYLATION OF PYRUVATE AND α -KETOGLUTARATE

Malic enzyme and oxalacetic carboxylase.—The reversible oxidative decarboxylation of a hydroxy acid by a pyridine nucleotide² may be termed an Ochoa reaction, in recognition of Ochoa's pioneering contributions to this problem. The first such reaction to be characterized was that which is catalyzed by the malic enzyme of pigeon liver (17), according to equation 1. Although this



² The following abbreviations will be used: TPN, triphosphopyridine nucleotide; TPN⁺, oxidized TPN; TPNH, reduced TPN; DPN, diphosphopyridine nucleotide; DPN⁺, oxidized DPN; DPNH, reduced DPN.

reaction may be considered to be the sum of two reactions; i.e., the oxidation of malate to oxalacetate (equation 2), and the decarboxylation of oxalacetate to pyruvate and carbon dioxide (equation 3), no free oxalacetate is formed as an intermediate and the total reaction is catalyzed by one enzyme. This enzyme



also catalyzes reaction 3 from left to right. Since purification studies of the pigeon liver enzyme indicated the two activities are inseparable, the conclusion has been drawn that one protein acts as a malic enzyme and as an oxalacetic carboxylase (17, 18). The protein does not catalyze reaction 2, however. This reaction is catalyzed by the classical malic dehydrogenase, which is a distinct enzyme reacting much more readily with DPN than TPN.

After the demonstration of the occurrence of the malic enzyme in animal tissues, its widespread distribution in the tissues of higher plants was established. Most of the earlier literature has been summarized in previous reviews [Goddard & Meeuse (3); Conn & Vennesland (19)]. Recently, a new simplified method of assay has been devised, by coupling the malic enzyme system with another enzyme, glutathione reductase; and with this procedure, the distribution studies have been considerably extended [Vennesland (20); Conn (21)]. No negative results were obtained with 15 different tissues and species tested.

There continues to be some uncertainty about the relationship of oxalacetic carboxylase activity to the malic enzyme. Veiga Salles & Ochoa (18) have pointed out that the animal enzyme acts as a carboxylase only at acid pH (with an optimum at pH 4.5). It is completely inactive as a carboxylase at pH 7.5, the optimum for the malic enzyme reaction. Older preliminary experiments with the parsley root oxalacetic carboxylase indicated, however, that the pH optimum was above 5.0, but these experiments were done with a crude preparation [Vennesland *et al.* (22)].

Fractionation studies of the plant malic enzyme and oxalacetic carboxylase from wheat germ have been conducted by Kraemer *et al.* (23). Because of the difficulty of assaying for malic enzyme in crude extracts, the oxalacetic carboxylase activity was used as the criterion for purification. Only partial purification was achieved, but this preliminary investigation indicated close association between malic enzyme and oxalacetic carboxylase activity just as in the case of pigeon liver. The wheat germ enzyme is not identical in all its properties with the animal enzyme, however. Perhaps the most striking difference lies in the response of the two oxalacetic carboxylase activities to TPN. This substance inhibits the wheat germ carboxylase, but activates the pigeon liver carboxylase (23, 24). Wheat germ contains a classical malic dehydrogenase as well as a malic enzyme. These enzymes can be distinguished

by their different behavior in the spectrophotometric test for reduction of TPN. Thus, in the presence of malic enzyme, malate reduces TPN only when Mn^{++} is added, and cyanide does not affect the reaction. In the presence of malic dehydrogenase, on the other hand, Mn^{++} has no effect on the reduction of TPN, but cyanide is required in order to bind the oxalacetate and thereby give an appreciable reaction.

The malic enzyme reaction is readily reversible, and when TPN, Mn^{++} , $C^{14}O_2$, malate and malic enzyme are incubated together, $C^{14}O_2$ enters the β -carboxyl carbon of malate. If malic dehydrogenase and oxalacetate are also present, the oxalacetate will likewise become labeled with C^{14} in the β -carboxyl carbon atom [Veiga Salles *et al.* (25); Ochoa (26)]. Purified malic enzyme alone does not, however, introduce $C^{14}O_2$ into oxalacetate. This might appear to raise some uncertainty about the mechanism of the Wood-Werkman reaction (equation 3; from right to left), since the main evidence for the occurrence of such a reaction rests on the demonstration of an exchange reaction in which $C^{14}O_2$ appears in the β -carboxyl group of oxalacetate. Fractionation studies of pigeon liver have demonstrated, however, that an enzyme or enzyme system independent of malic enzyme, and stimulated by ATP, can catalyze reaction 4 [Utter (27)]. McManus (28) has



also shown that an oxalacetic carboxylase preparation from *Micrococcus lysodeikticus* catalyzes reaction 4 independently of the malic enzyme system. A purified oxalacetic carboxylase from *Azotobacter vinelandii* does not catalyze an exchange reaction to any significant extent, however [Plaut & Lardy (29)]. Herbert (30) has achieved a high degree of purification of the lyso-deikticus enzyme, which contains no associated malic enzyme activity.

The findings cited show that the relationship of reactions 3 and 4 is not clear at present. Further complexities of the problem are discussed in detail in a review by Utter & Wood (31). In the case of the plant oxalacetic carboxylases, very little work has been done on the exchange reaction (22), and more extensive investigations are required before the problem of the occurrence and significance of the Wood-Werkman reaction in plants will be solved.

Considerable indirect evidence has accumulated to indicate that biotin may in some way affect β -decarboxylations. The effect is not clearly attributable to the action of biotin as a cofactor for the enzyme. Lichstein (32) has reviewed the extensive literature on the subject. Torda & Wolff (33) have reported that convulsive agents (acetyl choline, caffeine, camphor, cocaine, etc.) inhibit both the oxalacetic carboxylase and the pyruvic carboxylase of muskmelon pulp.

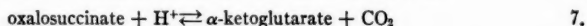
Byerrum *et al.* (34) have studied the effect of acetate ion concentration on the decarboxylation of oxalacetate catalyzed by the crystalline globulins of cucurbit seeds. An inhibition proportional to the cube of the ionic strength

was observed. They also found a small amount of Mn^{++} in the recrystallized protein, which suggested that the enzyme might be a metal protein like most other oxalacetic carboxylases. Calculation of a minimum molecular weight for the protein from their figures, however, gives a value of about fifty million, which appears very unlikely. It is improbable that these crystalline globulins function physiologically in the same way as the cation activated enzymes (22). They seem more closely related to some of the heat stable fractions of blood which decarboxylate oxalacetate and are independent of metal activators [Nossal (35)]. One of these blood components has been identified as the globin portion of hemoglobin. Perhaps the basic amino acid content of the proteins may be related to their catalytic effect.

Isocitric dehydrogenase.—Isocitric dehydrogenase has long been known as a TPN-specific enzyme which is stimulated by divalent cations. The reaction catalyzed is shown in equation 5. Working with animal tissues, Ochoa and his



collaborators demonstrated that this reaction occurs in two steps, as shown in equations 6 and 7. The first step occurs in the absence of a divalent cation.



The second step requires a divalent cation such as Mn^{++} . In a recent purification study of the enzyme from pig heart, Graffin & Ochoa (36) were unable to separate the enzyme catalyzing reaction 6 from the oxalosuccinic carboxylase, catalyzing reaction 7. This carries an implication that a situation exists similar to that found in the case of the malic enzyme. Reaction 5 is freely reversible and is another example of an Ochoa reaction. By analogy with the malic enzyme nomenclature, if reactions 6 and 7 are both catalyzed by one enzyme, it should perhaps be called an isocitric enzyme.

Whatley (37) has shown that isocitric dehydrogenase is present in leaves of chickweed, white deadnettle, elder, and charlock. The Chicago group has also extended its survey of the occurrence of this enzyme in plant material (20, 21). These studies confirm previous indications of a wide distribution for the enzyme in plant tissues.

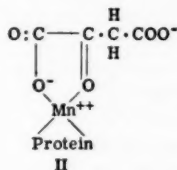
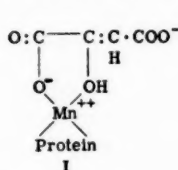
Until recently it appeared that isocitric dehydrogenase is always TPN-specific. Kornberg & Pricer (38) have found, however, that there are two distinct isocitric dehydrogenases in yeast. One of these reacts with TPN and is similar to the enzyme studied in animal tissues by Ochoa. The other isocitric dehydrogenase is DPN-specific, and requires adenylic acid as well as divalent cations for activity. Although this latter enzyme converts isocitrate to α -ketoglutarate and carbon dioxide, the reaction catalyzed does not appear to be reversible. Reduced DPN (DPNH) is not oxidized either by oxalosuccinate or by α -ketoglutarate and carbon dioxide, and the enzyme

does not decarboxylate oxalosuccinate. All isocitric dehydrogenases found in higher plants have hitherto been reported to be TPN-specific, but the occurrence of the DPN-specific enzyme in yeast raises the question of whether a similar enzyme might not be present in higher plants also.

Metal ion catalysis of β -decarboxylations.—The effect of metal ions on plant β -carboxylases has been reviewed by Hewitt (39). Metal ion activation of β -carboxylases, generally, is discussed further by Herbert (30). With the exception previously noted, all the β -carboxylases are activated by divalent metal ions. Monovalent and trivalent cations are largely without effect (see, however, p. 314). A number of divalent cations are good activators, but Mn^{++} is best. The relative effects of the ions appear to vary somewhat with the source of the enzyme, but the patterns are similar if not identical. Thus Mn^{++} , Cd^{++} and Co^{++} are the most effective activators of both parsley root oxaloacetic carboxylase [Speck (40)] and oxalacetic carboxylase from *Micrococcus lysodeikticus* [Herbert (30)]. Di- and trivalent cations also catalyze a nonenzymic decarboxylation of oxaloacetic and oxalosuccinic acids.

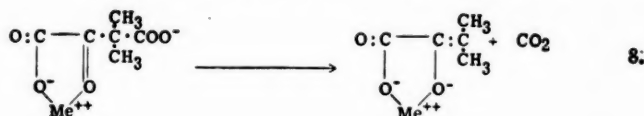
When oxalacetic and oxalosuccinic acids are decarboxylated enzymically, transient light absorption changes occur in the ultraviolet. The position of the bands indicates that enolates are formed [Kornberg *et al.* (41, 42)]. The changes are particularly marked with oxalosuccinic carboxylase and have been used as a basis of assay for the enzyme [Grafflin & Ochoa (36)]. The decarboxylation of these β -keto acids is also catalyzed by di- and trivalent cations in the absence of enzyme, and spectrophotometric changes likewise occur during the nonenzymic decarboxylation [Kornberg *et al.* (41, 42); Nossal (43)].

Kornberg *et al.* have suggested that the metal enolates of the β -keto acids are responsible for the changes in light absorption and have further postulated that the β -carboxylase enzymes act both by increasing the rate of such complex formation and by increasing the lability of the complex. According to this view, it is the enol form of the β -keto acids which is decarboxylated. Herbert (30) has postulated that a ternary, enzyme-metal-substrate complex is formed, for which he has suggested structure I.



The work of Steinberger & Westheimer (44, 45) throws considerable doubt, however, on the above interpretation of the spectrophotometric changes observed during the enzymic decarboxylation. These workers in-

vestigated the metal ion catalysis of the nonenzymic decarboxylation of dimethyl oxalacetate. This substance cannot enolize. It was shown that the keto form of the acid is converted to the enolate of the product, according to equation 8. The enolate subsequently ketonizes. Steinberger & Westheimer further suggested that the action of the metal ion be regarded as a model for the action of the enzyme. According to this view, the protein potentiates the metal ion effect, and the ternary enzyme-metal-substrate complex would have structure II. In the opinion of the reviewers, this suggestion is well founded. The evidence for reaction 8 is completely convincing, and it is reasonable that the decarboxylation of oxalacetate and of dimethyl oxalacetate should proceed by way of analogous intermediates. Enolates of oxalacetate would be formed, of course, but would not be expected to be decarboxylated, and the spectrophotometric changes should be ascribed to both the enolates of the substrate and to the enolate of the product.



Although monovalent cations have generally been reported to have no stimulating effect on malic enzymes, some exceptions to this rule should be noted. A stimulation of malic acid decarboxylation by potassium ions has been reported by Lwoff & Ionesco (46, 47), who worked with *Moraxella lwoffii*, and by Nossal (48) who worked with *Lactobacillus arabinosus*. The enzyme from *L. arabinosus* has been studied in some detail by Ochoa and his collaborators (49, 50). It is obviously related to the malic enzyme from plant and animal tissues but has some unique properties. Malic acid is converted to lactate and carbon dioxide. DPN and divalent cations are required as cofactors and potassium ions have a stimulating effect. The enzyme is formed adaptively, and oxalacetic carboxylase activity is associated with it. Free pyruvic acid does not appear as an intermediate during the reaction. Use of the malic enzyme from *L. arabinosus* as a tool for the specific and quantitative determination of malic acid has been described by Ochoa *et al.* (51) and by Nossal (52).

Photochemical coupling.—One of the most interesting findings of the past year has been the demonstration, by three different laboratories, that a mixture of chloroplasts, TPN, Mn^{++} , and malic enzyme will, on illumination, effect a conversion of pyruvate and carbon dioxide to malate. Vishniac & Ochoa (53, 54) employed malic enzyme from pigeon liver together with spinach chloroplasts. Tolmach (55, 56) employed malic enzyme from wheat germ, together with spinach chloroplasts, and Arnon (57) employed malic enzyme with washed chloroplasts. In Arnon's experiments enzyme and chloroplasts were both prepared from the same sources (sugar beet leaf and

sunflower leaf). The origin of the malic enzyme made no difference in the general nature of the results, a fact which further emphasizes the similarity of the enzyme reaction in very divergent living forms.

Vishniac & Ochoa identified and measured the malate formed by means of a specific enzyme from *L. arabinosus*. They also used $C^{14}O_2$ to show that the carbon dioxide actually was incorporated into malate, as did Tolmach. In addition to this, these authors have found that DPN, as well as TPN, was reduced by illuminated chloroplasts, and that other enzyme systems could function like the malic enzyme system. Enzymes tested included the isocitric system, lactic dehydrogenase, glutamic dehydrogenase, phosphoglyceraldehyde dehydrogenase, and hydrogenase (the enzyme from *Escherichia coli* catalyzing the reduction of fumarate to succinate by molecular hydrogen). Though none of these enzymes were prepared from plant sources, it seems only reasonable to assume, in view of Arnon's results, that all analogous enzymes if present in plants would behave in the same way.

Tolmach approached the problem by determining the effect of a large variety of added substances on oxygen evolution from illuminated spinach macerates. A number of substances, such as phosphoglycerate, pyruvate, and hexose diphosphate caused increased oxygen evolution from illuminated spinach juice, but by far the largest effect was obtained with TPN, which acted catalytically, in the sense that the amount of oxygen evolved was often many times greater, stoichiometrically, than would be expected from a photochemical reduction of the added TPN. Specific coupling with a carbon dioxide fixing reaction could not be proved in the juice preparations. When washed chloroplasts and the malic enzyme system were used, however, a coupling of oxygen evolution with carbon dioxide fixation occurred. DPN also increased oxygen evolution when added to spinach juice, but the results were not as striking as those obtained with TPN. The reactions occurring in Tolmach's system are undoubtedly complex, as indicated by the kinetics of the oxygen evolution. Complexity is to be expected, in view of the variety of enzymes which must be present in the unfractionated spinach juice.

Arnon emphasized the fact that the malic enzyme is present as a soluble protein in the cytoplasm, and not in the washed chloroplasts. Chloroplasts alone could not be expected, therefore, to effect a reductive carboxylation of pyruvate on illumination. Since the malic enzyme is very widely distributed, however, and has been found in all green leaves tested to date, one may expect photochemical reductions of this nature in green leaves generally.

The cofactor, TPN, has also been shown to be present in green leaves. Whatley (58) found it in concentrations of about 2 to 8 $\mu\text{g.}$ per gm. fresh weight in leaves of clover, runner bean, elder, chickweed, oat, and potato. Anderson (59) has obtained similar results with spinach leaf and cabbage. TPN is not confined to leaves, but occurs in other parts of the plant as well. Wheat germ and avocado contained 32 and 20 $\mu\text{g.}/\text{gm.}$ fresh weight, respectively. In this connection it is interesting that Whatley found only traces of

DPN in green leaves, although other plant tissues contain more DPN than TPN, as has been found to be the case for animal tissues. One is tempted to speculate that there may be a connection between this fact and the failure of Tewfik & Stumpf (60) to find an active DPN glyceraldehyde dehydrogenase in mature pea leaves.

Physiological function.—It has been established beyond any reasonable doubt that the dicarboxylic acids may be formed from carbohydrate in plant tissues, by a carboxylation of pyruvate. This is particularly clear in the case of the succulents, which show the characteristic diurnal variation in organic acid content. Evidence from studies of the intact plant has been summarized in reviews by Thomas (61) and by Wolf (62).

β -carboxylations are not limited to succulents but occur in other plants also. Gibbs has studied the incorporation of $C^{14}O_2$ into carbohydrate and malate in sunflower leaves (63), and found that the distribution of C^{14} in these compounds after prolonged dark exposure is identical with the distribution found in animal tissues. The C^{14} enters only the carboxyl groups of malate, and the 3, 4 positions of hexose. This is the distribution to be expected if the C^{14} enters the C_4 acids by a carboxylation reaction, is equilibrated in the carboxyl groups through the formation of a symmetrical acid, and then enters carbohydrate by way of carboxyl-labeled pyruvate by the conventional glycolytic mechanism or a similar path. No net uptake of carbon dioxide is involved.

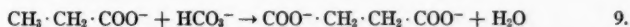
Stutz & Burris (64) exposed various plants (*Bryophyllum calycinum*, tomato, tobacco, barley, and rhubarb) to $C^{14}O_2$ for varying periods of time, both in the light and in the dark. The organic acids from the leaves were then separated on a silica gel column, and their specific radioactivities were determined. The results obtained varied somewhat among the different species, but the patterns were similar. In all cases, malic acid contained the bulk of the C^{14} fixed in the plant acids, and also had the highest specific activity. The incorporation of C^{14} into the tricarboxylic acids was often quite slow, and the data indicated that, although the acids might very well be interconverted by the reactions of the Krebs cycle, such a cycle could only function relatively slowly. The slow incorporation of C^{14} into the tricarboxylic acids as compared with the incorporation into malic acid, may seem to contradict the evidence that both isocitric dehydrogenase as well as malic enzyme are usually present in plants in abundant quantity. It should be recalled, however, that the rate of the carboxylation reactions is probably a function not only of enzyme concentration and carbon dioxide tension, but also of the concentration of the α -keto acid. It is possible that the rate of formation of isocitrate is slow because the concentration of α -ketoglutarate may be kept low. In any event, tricarboxylic acids are formed also by condensation of a C_2 unit with oxalacetate, and it is consequently difficult, at present, to assess the functional importance of the reversibility of the oxidative decarboxylation of isocitrate.

The question of whether the carboxylation of pyruvate to a C_4 dicarboxylic acid plays an obligatory role in the photosynthetic formation of carbohydrate is more difficult to answer. On the whole, the bulk of the present evidence indicates that, if this is the case, the C_4 acid is not malic acid in equilibrium with the malic acid of the cell sap. Stutz & Burris (64) concluded that none of the organic acids are early products of photosynthesis, since the specific radioactivity of all the organic acids in a leaf increased in the dark in the absence of C^{14} after a preliminary illumination period in the presence of C^{14} . Calvin & Benson and their associates introduced malic acid in one of their working hypotheses for the path of carbon in photosynthesis and later discarded it in favor of a related hypothetical C_4 acid (65). It is not yet clear what place malic acid, or a hypothetical C_4 acid, occupies in their more recent working hypothesis (see p. 321). Gaffron, Fager & Rosenberg (2, 66) have consistently maintained the view that a C_4 acid is not on the direct path of carbon in photosynthesis. The question of the origin of the C_2 unit (the precursor for phosphoglycerate) is at the crux of the problem and is discussed further in a later section (p. 318).

Meanwhile it should be emphasized that the ability of illuminated chloroplasts to effect a reductive carboxylation of pyruvate by coupling with the malic enzyme system does not afford an answer to the problem of the formation of the C_2 compound in photosynthesis. The artificial systems recently studied are using light energy to convert carbohydrate (pyruvate) to organic acids. This can be accomplished by any system capable of reducing TPN, and occurs normally in the dark as well as in the light [Ochoa & Vishniac (54); Ochoa *et al.* (51)].

FORMATION OF SUCCINATE FROM PROPIONATE AND CARBON DIOXIDE

A new type of photosynthetic reaction has recently been demonstrated by Larsen (67). Studies of the photosynthetic green sulfur bacteria, *Chlorobium thiosulphatophilum*, showed that this organism, in the light, could form succinic acid from propionic acid and carbon dioxide. When thiosulfate was omitted from the medium, no growth occurred, and the major over-all reaction taking place could be represented by equation 9. When the organism was allowed to photosynthesize



in the presence of both thiosulfate and propionic acid, the rate of carbon dioxide fixation was equal to the sum of the rates observed with thiosulfate and propionate separately. This was regarded as partial evidence for the occurrence of more than one carbon dioxide-fixing mechanism.

In his discussion of the mechanism of formation of succinate from propionate, Larsen considers two possible reaction paths. One path involves an oxidation of propionate to pyruvate, followed by reductive carboxylation of

pyruvate to malate, and reduction of malate to succinate. Most of the reactions in this scheme are known to occur in many living organisms. However, the occurrence of a direct interconversion of propionate and pyruvate by oxidation and reduction is still open to question. As an alternative path, the direct carboxylation of propionate, or of some active form of propionate to succinate, is also considered. Barban & Aji (67a) have presented evidence that such a reaction may occur in *Propionibacterium pentosaceum*. The energy required for the postulated reaction (equation 9) is such that a decarboxylation of succinate would occur unless coupling with an energy-yielding mechanism is provided.

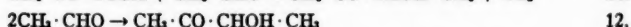
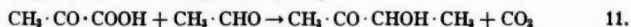
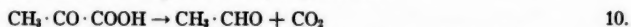
Some evidence for a direct decarboxylation of succinate is also available in the case of bacteria. Johns (68) showed that washed suspensions of *Veillonella gazogenes* converted succinate to carbon dioxide and propionate. The amount of propionate formed from a substrate like lactate was dependent on carbon dioxide tension. Delwiche (69) showed that suspensions of *Propionibacterium pentosaceum* decarboxylated succinate. The fact that the reaction was not inhibited by the presence of .01 *M* semicarbazide was regarded as evidence that oxidation of succinate was not necessary for decarboxylation.

Anomalies in propionate metabolism in animal tissues have been discussed recently by Lorber *et al.* (70) [see also Utter & Wood (31)]. The suggestion that the interconversion of pyruvate and propionate can occur only by way of the dicarboxylic acids fits many available facts. It seems, however, that a direct demonstration of the occurrence of reaction 9 from right to left in cell-free extracts is necessary before a conclusion can finally be drawn that such a reaction actually takes place.

ORIGIN AND METABOLISM OF C₂ UNITS

Because of the apparent central importance of the metabolism of C₂ units in connection with photosynthesis, an attempt is made in this section to summarize some known facts regarding plant enzyme systems which can form and metabolize C₂ compounds.

Decarboxylation of pyruvate.—Pyruvic carboxylase is widely distributed in the tissues of higher plants. The pyruvic carboxylase of wheat germ has been purified by Singer & Pensky (71). In a preliminary note, these authors report that the enzyme is very similar to the carboxylase of yeast and requires diphosphothiamine and Mg⁺⁺ for activity. Purification of 11,000-fold was obtained, and the activity of the product was about four times greater than that of the most active preparation ever obtained from yeast. The purified enzyme was reported to catalyze the synthesis of acetoin from pyruvate and acetaldehyde. Acetoin synthesis also occurred, though more slowly, from acetaldehyde alone. The enzyme can apparently catalyze the three reactions shown in equations 10, 11, and 12.



The pH optima and the cofactor requirements were the same for all the reactions. Furthermore, the relative values of the three reaction rates remained constant on purification and after a variety of partial inactivation procedures. The conclusion was drawn that one protein catalyzed all the reactions.

In some bacteria, α -acetolactate has been shown to be an intermediate in acetoin formation from pyruvate (72, 73). This has not been shown to be the case for higher plants. In view of the results with wheat germ carboxylase, it seems that the pyruvic carboxylase may be responsible for the long known ability of some plant preparations to form acetoin. The literature on the general problem of the mechanism of acetoin formation has been summarized in two reviews [Ochoa (74); Vennesland (75)].

The acetaldehyde formed from pyruvate by pyruvic carboxylase is undoubtedly the immediate precursor of ethanol, the end product of anaerobic fermentation. It is an open question, however, whether free acetaldehyde is formed as an intermediate in the conversion of pyruvate to the C_2 units which may be assumed to be involved both in fat formation and in respiratory breakdown over the Krebs cycle. The mitochondria of Miller *et al.* (6) catalyze an oxidative decarboxylation of both pyruvate and α -ketoglutarate, but virtually no information is available yet about the detailed mechanism of these reactions, or about the possible involvement of coenzyme A and the "pyruvate oxidation factor" (76, 77) in pyruvate metabolism in plants. The mechanism of citrate formation from pyruvate and oxalacetate in animal tissues and the function of coenzyme A in the process have been beautifully elucidated by Ochoa and his collaborators (78 to 81).

Formation of phosphoglyceric acid.—There remains general agreement that the first identified, stable locus of carbon dioxide fixation in photosynthesis at medium to high light intensities is the carboxyl group of phosphoglyceric acid. Carbon dioxide fixation in this position can, of course, occur by way of the dark fixation path described before (pp. 309, 316). This is not the mechanism of the fixation in the light, however, since pyruvate is not the precursor of the phosphoglycerate [Gaffron & Fager (2)]. It appears that some C_2 unit is carboxylated directly to yield phosphoglyceric acid or a precursor thereof. The origin and identity of this C_2 unit and the mechanism of its carboxylation constitute some of the major problems currently under investigation. One may anticipate that the chemical detail of this key carboxylation reaction will resist analysis as long as studies are restricted to intact cells. It is encouraging therefore, that Fager (82) has been able to obtain a cell-free macerate of spinach, which is capable of fixing carbon dioxide into phosphoglyceric acid. On incubation of this preparation with $C^{14}O_2$ in the dark, the C^{14} was introduced into several compounds, with 65 per cent of the total in phosphoglyceric acid. Illumination of the identical incubation mixture resulted in an approximate doubling of the total carbon dioxide fixation, with 60 per cent of the C^{14} in phosphoglyceric acid. The fixation in phosphoglyceric acid was therefore increased by light almost to

the same extent as the total fixation. Furthermore, the labeling of phosphoglyceric acid preceded the labeling of pyruvate, just as was found to be the case for living cells.

Recent results of Aronoff (83) indicate that there may be more than one type of carboxylation reaction of C_2 units to glycerate. Using a procedure designed to minimize hydrolysis during isolation, he concluded that in soy bean leaves the major product of 5 sec. illumination with tracer under conditions of steady state photosynthesis was glyceric acid, with smaller amounts of 2- and 3-phosphoglyceric acid, and 2,3-diphosphoglyceric acid. After exposure of leaves to $C^{14}O_2$ for 15 min. in the dark, followed by 5 sec. of illumination, the three types of glyceric acid contained different distributions of C^{14} . All had most of the label in the carboxyl group, but glyceric acid contained no label in the β carbon and 14 per cent of the label in the α carbon, diphosphoglycerate contained more C^{14} in the β than in the α carbon, and in 2- and 3-phosphoglyceric acids the label was equally distributed in the α and β carbons. The conclusion was drawn that the acids must arise by independent carboxylation reactions. No figures were given for the C^{14} distribution in these acids after dark exposure alone. The data were also interpreted as additional evidence against the occurrence of a $C_1 + C_1$ condensation in photosynthesis.

The dicarboxylic acid cycle.—Recent studies of the metabolism of molds, bacteria, and yeast have provided evidence for the existence of a so-called "dicarboxylic acid cycle" as an alternate path to the citrate cycle in the respiration of carbon compounds. Such a reaction sequence is thought in some cases to coexist with the cycle, in others to replace it. The subject has been reviewed by Aji (84).

The key reaction of the dicarboxylic acid cycle is the condensation of two C_2 acids to form a dicarboxylic acid. It is usually postulated that the C_2 units are acetate and the C_4 acid is succinate. The reaction, then, is the Thunberg-Knoop condensation, which must proceed with an oxidative removal of two hydrogen atoms according to equation 13. The oxidative reconversion of succinate



to acetate can subsequently occur by way of fumarate, malate, oxalacetate, and pyruvate by well-established reactions, thus accounting for the complete oxidation of acetate. There is no evidence for the occurrence of a Thunberg-Knoop condensation in higher plants, and in the case of yeast, the evidence for its occurrence is largely indirect. In bacteria and molds, experimental evidence is stronger [Aji (84); Lewis & Weinhouse (85); see, however, Utter & Wood (31), p. 117].

The question of the occurrence of a Thunberg-Knoop condensation is of interest, not only as an alternate mechanism to the Krebs cycle, but also because it has been suggested that a dicarboxylic acid may split to two C_2

units which may serve as precursors of phosphoglyceric acid in photosynthesis (1). No direct experimental evidence for the occurrence of such a cleavage in higher plants has yet been obtained, though in molds it has been suggested that oxalacetate may split to oxalate and acetate, on the basis of studies of the eventual distribution of $C^{14}O_2$ into oxalate and citrate [Lewis & Weinhouse (86)].

Sedoheptulose and ribulose.—Benson, Bassham & Calvin (87) have reported the identification of phosphate esters of the seven carbon ketose, sedoheptulose, as one of the labeled products of short-term photosynthesis studies carried out with $C^{14}O_2$. The early labeling, and the fact that septose was found in all plants studied (*Chlorella*, *Scenedesmus*, *Rhodospirillum rubrum*, and the leaves of barley seedlings, soybean, alfalfa, sugar beet, spinach, and geranium), suggested a role as an intermediate in photosynthesis. Benson (88) also identified phosphate esters of the five carbon ketose, ribulose, among the photosynthesis products. It was accordingly suggested that the septose was formed by condensation of a C_3 unit and a C_4 dicarboxylic acid such as malate, and that it then splits off a C_2 unit to form a pentose which, in turn, splits off a C_2 unit to regenerate triose (89). In this scheme both sedoheptulose and ribulose are precursors of the C_2 unit which is carboxylated to phosphoglycerate during photosynthesis. Very little is known about the metabolism of sedoheptulose. It accumulates in large quantities in some Crassulacean species which also accumulate malate. An early suggestion that it functioned there as a malate precursor [Bennet-Clark (90)] was later discarded, since malate accumulation was not accompanied by a decrease in the concentration of the seven carbon sugar [cited by Bonner (91)].

Sedoheptulose was first identified as the main soluble sugar of *Sedum spectabile*. Its discovery and the elucidation of its chemical structure were mainly the work of Hudson and his associates (92, 93, 94). Klevstrand & Nordal (95) have developed a spraying reagent for paper chromatograms which is specific for ketoseptoses, and which was applied by Nordal & Klevstrand (96) to show that such a sugar was present in all of the seven species of Crassulacean plants tested. The specificity of the color test has been studied further by Bevenue & Williams (97). The demonstration that sedoheptulose is widely distributed and undergoes rapid turnover during photosynthesis should stimulate intensive studies of the metabolism of this compound. Meanwhile, its role in photosynthesis must be regarded as speculative.

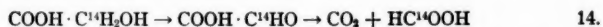
In the case of ribulose, we have relatively more information available about its metabolic origin and fate than in the case of sedoheptulose. Thus, ribulose-5-phosphate has been identified as a product of glucose-6-phosphate oxidation in yeast and bacteria (see pp. 323, 325). Also, ribulose-5-phosphate may be isomerized to ribose-5-phosphate. There is considerable experimental evidence, also, that pentose may undergo a reversible cleavage to

$C_3 + C_2$ by the action of an enzyme similar to aldolase. The literature on the subject is reviewed by Horecker *et al.* (98). The C_2 unit formed directly from pentose may be glycolaldehyde or glycolaldehyde phosphate [Marmur & Schlenk (99)]. Such C_2 units are regarded as closely related to the C_2 unit which is carboxylated to glyceric acid in photosynthesis (1, 2). The work on pentose metabolism has been limited to microorganisms and animal tissues, and information about similar metabolic pathways in higher plants is urgently needed.

Glycolic acid metabolism.—The possibility that glycolic acid is a precursor of glyceric acid in photosynthesis (1, 2) has received further support from the results of Schou *et al.* (100). The metabolism of labeled glycolic acid by *Scenedesmus* was studied aerobically and anaerobically, both in the light and in the dark. At pH 3.8, appreciable assimilation occurred. In the dark, anaerobically, a large proportion of the isotope entered serine and glycine. The phosphoglyceric acid formed in the light was isolated and degraded. The label was found to be equally distributed in the α and β carbons after administration of $1-C^{14}$ or $2-C^{14}$ glycolic acid. Accumulation of glycolic acid in the light in some plant species was also noted by Stutz & Burris (64) in their study of $C^{14}O_2$ incorporation into plant acids (discussed on p. 316).

Burris and his collaborators have continued their investigation of the metabolism of glycolic acid in plants (101, 102, 103). The enzyme oxidizing glycolic acid is present only in the green parts of plants. It is absent in roots and in etiolated shoots. When intact etiolated barley is exposed to light, rapid activation of the enzyme occurs within 6 hr. This activation is not proportional to the rate of chlorophyll formation. The enzyme does not disappear when green plants are kept in the dark.

The first product of glycolic acid oxidation is glyoxylic acid. Glyoxylic acid is oxidized by another enzyme system which is not light-activated. When glycolic acid was oxidized by unpurified tobacco sap, the oxygen uptake was greater than two atoms per mole of glycolic acid, chlorophyll was bleached in the process, and the main products of the reaction were not identified. A partially purified, lyophilized enzyme preparation from tobacco leaves, however, oxidized glycolic acid, through glyoxylic acid, to formic acid and carbon dioxide. Tests with glycolic acid labeled with C^{14} showed that the carboxyl group yielded carbon dioxide, and the α carbon was the source of formate, as shown in equation 14.



Information is lacking about the mechanism of the oxidative decarboxylation of glyoxylic acid.

Glycolic acid may have a role in animal as well as in plant metabolism. Örström (104) has identified phosphoglycolic acid as a constituent of human blood.

The formation of formate from glycolic acid, and the conversion of gly-

colic acid to serine and glycine, are all reactions which deserve attention in connection with the elucidation of the path of labeled carbon in any intact metabolizing system. The conversion of the α carbon of glycine to the β carbon of serine, by way of an active C_1 unit which is not carbon dioxide, and the reconversion of serine to glycine by the removal of the β carbon as a similar C_1 unit are reactions known to occur in animal tissues, and they very probably occur in plants also. Since glyceric acid and serine are also closely related, it may be noted that the α carbon of glyceric acid may enter the β position of the same compound by becoming, successively, the α carbon of serine, the α carbon of glycine, the β carbon of serine, and the β carbon of glyceric acid. Such reactions might have a net result similar to that given by the formation of a symmetrical C_2 precursor of glyceric acid. Full treatment of this subject is outside the scope of this review, but reference may be made to the review of Utter & Wood (31), and to a very stimulating article by Wood (105) on the role of C_1 compounds in metabolism. Wood points out that, because of its recently demonstrated metabolic function in animal tissues, formaldehyde will almost certainly "once more receive serious consideration in photosynthesis, though probably not in terms of the old proposal of a carbon-by-carbon build up of sugars by aldol condensation of formaldehyde."

There is no evidence that active C_1 units related to formate or formaldehyde can be formed in plants directly from carbon dioxide. Plants contain an enzyme, formic dehydrogenase, which oxidizes formate to carbon dioxide, but the oxidation appears to be irreversible. Matthews & Vennesland (106) have obtained a partially purified formic dehydrogenase from peas, and Davison (107) has purified the enzyme from bean seeds. The enzyme is specific for DPN and catalyzes the reaction shown in equation 15. The irreversibility of this reaction,



which could be predicted from thermodynamic considerations, was tested with $C^{14}\text{O}_2$. No evidence was obtained of any appreciable exchange of $C^{14}\text{O}_2$ with formate.

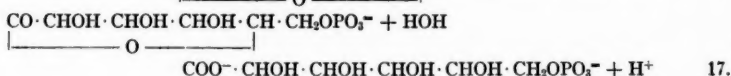
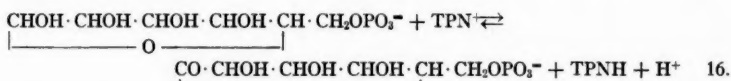
THE OXIDATIVE DEGRADATION OF GLUCOSE-6-PHOSPHATE

An enzyme or enzyme system catalyzing the oxidation of glucose-6-phosphate by oxidized TPN (TPN^+) has recently been demonstrated in roots, leaves, and seeds of a wide variety of higher plants [Conn & Vennesland (108); Conn (21)]. The mechanism of the reaction sequence in plant tissues has not yet been studied; but carbon dioxide is known to be formed [Vennesland (20)]; and it seems probable that the enzyme systems involved are similar to those of yeast, bacteria, and animal tissues.

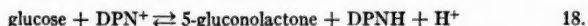
The direct oxidation of carbohydrate by a path distinct from the glycolytic or fermentative route has long been known to occur in yeast. The classi-

cal work of Warburg on "Zwischenferment" or glucose-6-phosphate dehydrogenase established that the reaction catalyzed by this enzyme consisted of an oxidation of glucose-6-phosphate to phosphogluconic acid by TPN^+ .

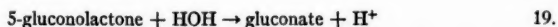
Cori & Lipmann (98, 109) have investigated the mechanism of this reaction and come to the conclusion that the primary reaction product is phosphogluconolactone, which is subsequently hydrolyzed to phosphogluconate. The two reactions are shown in equations 16 and 17.



The possible occurrence of reaction 16 was suggested by the fact that gluconolactone is the immediate product of the nonenzymatic oxidation of glucose by bromine [Isbell & Hudson (110)] and also of the enzymic oxidation of glucose by the mold flavoprotein, notatin [Bentley & Neuberger (111)]. Strecker & Korkes (112) have likewise found that glucose dehydrogenase from beef liver, a DPN enzyme, converts glucose to gluconolactone. They showed further that this reaction was reversible (equation 18).



The over-all conversion of glucose to gluconate ion showed only slight reversibility, however, although a small amount of back reaction could be demonstrated at fairly low pH. The largest energy change in the conversion of glucose to gluconate occurs, therefore, in the hydrolysis of the lactone to the gluconate ion (equation 19).

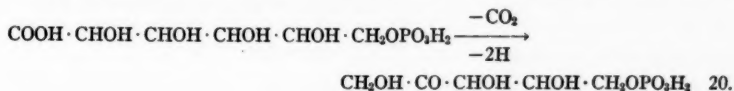


Lipmann has pointed out that reaction 16 would be expected to be reversible and the results of Strecker & Korkes may be regarded as a partial confirmation of this prediction, in view of the close similarity between reactions 16 and 18.

There is general agreement that the further oxidative degradation of phosphogluconate yields pentose phosphates and carbon dioxide. The reaction is regarded as a possible source of the ribose of nucleic acids. In order to convert 6-phosphogluconate into ribose-5-phosphate, an oxidative removal of 2H atoms, a decarboxylation, and a change of configuration of carbon atom number three of the gluconate molecule are required. Various mechanisms have been proposed for these conversions. The reaction mechanism proposed by Horecker and his collaborators (98, 113, 114, 115) appear at present to have the best experimental support, since the work has been

done with relatively pure enzyme preparations. The starting material was yeast.

Horecker has identified ribulose-5-phosphate as a product of an enzymic oxidative decarboxylation of phosphogluconic acid according to equation 20.



The oxidizing agent is TPN^+ . The analogy to the enzymic oxidative decarboxylation of malate and isocitrate is pointed out. Such a reaction, if catalyzed by one enzyme, may be considered as a new example of an Ochoa reaction.

Horecker has shown further that C^{14} added as C^{14}O_2 is incorporated in the phosphogluconic acid during the reaction. In other words, the reaction appears to be reversible, as might be expected of an oxidative β -decarboxylation. The ribulose-5-phosphate is subsequently converted to ribose-5-phosphate, an equilibrium mixture of the two sugars being obtained.

Cohen & McNair Scott (98, 116 to 120) have studied the oxidation of glucose-6-phosphate primarily by preparations from *E. coli*. They suggest that a 1,2 enediol pentose-5-phosphate may be the primary decarboxylation product, and recall, in this connection, a suggestion of Veiga Salles & Ochoa (18) that an enol may be formed during the oxidative decarboxylation of malate. The decarboxylation mechanism of Steinberger & Westheimer (pp. 313-14) should be called to mind in this connection.

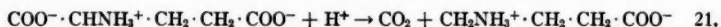
Dickens & Glock (121, 122) have shown that the direct oxidative degradation of glucose-6-phosphate occurs very generally in animal tissues, with pentose as a product. They postulate a conversion sequence of phosphogluconic acid to phosphogluconolactone, a dehydrogenation to 6-phospho-2 (or 3) ketogluconolactone, an enolization, an opening of the lactone ring, and finally a decarboxylation to D-ribose-5-phosphate. The question of whether the decarboxylation is α or β is here left open.

In view of the demonstrated reversibility of some of the reactions just described, one might expect incorporation of carbon dioxide carbon into the C_1 position of glucose. The reviewers know of no evidence that such incorporation occurs to an appreciable extent in any living organisms. Since the equilibrium of reaction 17 presumably lies far to the right, the conversion of phosphogluconate to phosphogluconolactone would not be expected to occur at an appreciable rate at neutral pH. The practical irreversibility of reaction 17 therefore would account for the irreversibility of the over-all reaction.

The formation of ribulose-phosphate from glucose-6-phosphate is of interest because of the postulated function of the pentose as a source of C_2 units in photosynthesis. This question and the question of the mechanism of pentose breakdown are discussed on pp. 321-22.

AMINO ACID DECARBOXYLASES

Schales has reviewed the subject of amino acid decarboxylases (123). These enzymes are present in animal tissues and bacteria, and have been studied mainly in the latter organisms. The only amino acid decarboxylase which has been positively identified in higher plants is glutamic acid decarboxylase. This is a pyridoxal phosphate protein which converts glutamic acid to amino butyric acid and carbon dioxide according to equation 21.



The first evidence for the existence of such an enzyme in higher plants was provided in 1937 by Okunuki (124), who showed that an enzyme causing a decarboxylation of L-glutamate was present in the pollen and bulb of *Lilium auratum*, and in beets, cabbage, radishes, spinach, and carrots. The enzyme of lily pollen was particularly active and was studied further (125). It was inhibited by cyanide, hydroxylamine, hydrazine, semicarbazide, and other carbonyl group reagents. Cyanide was particularly effective; $0.35 \times 10^{-5} M$ KCN gave 50 per cent inhibition. The enzyme did not act on D-glutamic acid or on acetylglutamic acid.

Schales and his associates (126, 127) extended these investigations and found that glutamic decarboxylase was very widely distributed in higher plants. Over 30 different sources were identified. Okunuki had thought the enzyme was insoluble, but Schales found it could often be readily solubilized. The enzyme from carrot was selected for more detailed studies. Its kinetics were investigated in detail. Pyridoxal phosphate was shown to act as a co-enzyme. The decarboxylase from squash has been used as a reagent for the quantitative determination of L-glutamic acid [Schales & Schales (128)].

Morrison (10) found a very active glutamic decarboxylase in rhubarb leaves. Leaf homogenate caused a carbon dioxide evolution from added glutamate approximately equivalent to that obtained with leaf disks. Activity was associated with the insoluble portion of the homogenate. The enzyme was inactivated on dialysis and inhibited by cyanide.

Werle & Brüninghaus (129) and Hasse & Schumacher (130) studied the action of an enzyme preparation from winter radish on glutamic acid. The latter workers employed the washed pulp of the plant as the enzyme preparation. The product of the reaction was isolated and identified as γ -amino butyric acid.

Beevers (131) reported the presence of an active glutamic acid decarboxylase in barley roots. This enzyme could be obtained as an aqueous extract, from which it was precipitated with $(\text{NH}_4)_2\text{SO}_4$ in stable form. The kinetics of the reaction were studied. The decarboxylation of glutamate was almost complete and the product was identified as γ -amino butyric acid.

Not only is the enzyme widely distributed, but its decarboxylation product has also been found as a normal constituent of plant extracts. This was first shown by Steward *et al.* (132, 133), who identified γ -amino butyric acid

among the components of the soluble nitrogen compounds of potato tubers. It was also found in alfalfa leaves, though in smaller amounts (134). Westall (135) isolated γ -amino butyric acid from beet root, and estimated it was present in a concentration of 0.02 per cent on a fresh weight basis. Hulme & Arthington (136) likewise found it in apple pulp, where it constituted a considerable proportion of the soluble nitrogen.

The function of the decarboxylase, and its decarboxylation product is still unknown. Steward (133) has suggested that γ -amino butyric acid might function as a precursor (by carboxylation), instead of a product of glutamic acid. The decarboxylase activity of potato tubers was very low; and γ -amino butyric acid disappeared when protein was synthesized in potato slices, but could not be identified as a constituent of the protein.

No direct studies have been made of the possible reversibility of the enzyme reaction in plants. However, Hanke & Siddiqi (137) have found that bacterial lysine decarboxylase and tyrosine decarboxylase cause an exchange of $C^{14}O_2$ into the carboxyl groups of their respective substrates, and Koppelman, Mandeles & Hanke (138) have found that glutamic decarboxylase from *Clostridium welchii* behaves similarly. One might therefore expect that the plant enzyme can also cause an incorporation of $C^{14}O_2$ into the carboxyl group of glutamic acid.

Another possibility for the metabolic function of γ -amino butyric acid may, however, be considered. If this compound is formed from glutamate which in turn is derived from α -ketoglutarate synthesized from labeled precursors by way of the Krebs cycle, then the γ -amino butyric acid would have the same isotope distribution as succinic semialdehyde or a succinyl derivative derived by decarboxylation of α -ketoglutarate. Shemin & Wittenberg (139) have shown that the pyrrol rings of heme are formed from a four carbon precursor of this sort, and Granick (140) has pointed out that chlorophyll synthesis and heme synthesis are expected to proceed by a common initial path. It does not appear unreasonable, therefore, that the widely distributed glutamic decarboxylase in higher plants might function to provide γ -amino butyric acid as a chlorophyll precursor.

The presence of β -alanine (133, 135, 136) in plant extracts implies the existence of an aspartic acid decarboxylase, but direct demonstration of such an enzyme in plant extracts has not been achieved. Similarly, formation of histamine is indirect evidence for a histidine decarboxylase and of hydroxytyramine for a dopa decarboxylase, but these enzymes likewise have not yet been demonstrated directly [Werle & Raub (141)].

CONCLUSION

Future investigations will undoubtedly reveal that there are many more carboxylases in plants than those discussed in the present chapter. Some topics, such as the function of carbon dioxide in purine synthesis, have been completely omitted, because the process has not been studied, as yet, in

higher plants. One may anticipate, however, that investigators in this field will find the ground work laid by those who are now working with animal tissues and microorganisms (142).

The subject of carbon dioxide fixation has attracted much current interest, as reflected by the numerous recent reviews which have appeared. Attention is particularly directed to the following references. The papers presented at two symposia on "Carbon Dioxide Fixation and Photosynthesis" are available in published form (143, 144). Detailed and authoritative reviews are also available on "Fixation of Carbon Dioxide" [Utter & Wood (31)] and "Biological Mechanisms of Carboxylation and Decarboxylation" [Ochoa (74)]. In addition, chapters in a recent reference work cover the keto acid carboxylases [Vennesland (75)], the amino acid decarboxylases [Schales (123)], and enzymatic mechanism of carbon dioxide fixation [Ochoa (145).]

LITERATURE CITED

1. Benson, A. A., and Calvin, M., *Ann. Rev. Plant Physiol.*, **1**, 25-42 (1950)
2. Gaffron, H., and Fager, E. W., *Ann. Rev. Plant Physiol.*, **2**, 87-114 (1951)
3. Goddard, D. R., and Meeuse, B. J. D., *Ann. Rev. Plant Physiol.*, **1**, 207-32 (1950)
4. Krebs, H. A., *Harvey Lectures Ser.* **44**, 165-99 (1948-49)
5. Krebs, H. A., *Ciba Foundation Conf. Isotopes Biochem.*, 225-26 (Wolstenholme, G. E. W., Ed., J. & A. Churchill, Ltd., London, England, 1951)
6. Miller, A., Bonner, J., Axelrod, B., and Bandurski, R., *Proc. Natl. Acad. Sci. U. S.*, **37**, 855-62 (1951)
7. Stafford, H. A., *Physiol. Plantarum*, **4**, 696-741 (1951)
8. Price, C. A., and Thimann, K. V., *Arch. Biochem. Biophys.*, **33**, 170-71 (1951)
9. Hasse, K., and Fruhstorfer, W., *Naturwissenschaften*, **37**, 399-400 (1950)
10. Morrison, J. F., *Australian J. Exptl. Biol. Med. Sci.*, **28**, 311-20 (1950)
11. Boswell, J. G., *Ann. Botany*, **14**, 521-43 (1950)
12. Barrón, E. S. G., Link, G. K. K., Klein, R. M., and Michel, B. E., *Arch. Biochem.*, **28**, 377-98 (1950)
13. Foulkes, E. C., *Biochem. J.*, **48**, 378-83 (1951)
14. Eny, D. M., *Plant Physiol.*, **26**, 268-89 (1951)
15. Stutz, R. E., and Burris, R. H., *Plant Physiol.*, **26**, 226-43 (1951)
16. Laties, G., *Organic Acid Metabolism in Higher Plants* (Goddard, D. R., Ed., Iowa State College Press, Ames, Iowa, in press, 1952)
17. Ochoa, S., Mehler, A. H., and Kornberg, A., *J. Biol. Chem.*, **174**, 979-1000 (1948)
18. Veiga Salles, J. B., and Ochoa, S., *J. Biol. Chem.*, **187**, 849-61 (1950)
19. Conn, E. E., and Vennesland, B., *Brookhaven Conf. Rept., BNL 70 (C-13)*, 64-76 (1950)
20. Vennesland, B., *Organic Acid Metabolism in Higher Plants* (Goddard, D. R., Ed., Iowa State College Press, Ames, Iowa, in press, 1952)
21. Conn, E. E., *Organic Acid Metabolism in Higher Plants* (Goddard, D. R., Ed., Iowa State College Press, Ames, Iowa, in press, 1952)
22. Vennesland, B., Gollub, M. C., and Speck, J. F., *J. Biol. Chem.*, **178**, 301-14 (1949)
23. Kraemer, L. M., Conn, E. E., and Vennesland, B., *J. Biol. Chem.*, **188**, 583-91 (1951)
24. Vennesland, B., Evans, E. A., Jr., and Altman, K. I., *J. Biol. Chem.*, **171**, 675-86 (1947)
25. Veiga Salles, J. B., Harary, I., Banfi, R. F., and Ochoa, S., *Nature*, **165**, 675 (1950)
26. Ochoa, S., *Symposia Soc. Exptl. Biol.*, **5**, 29-51 (1951)
27. Utter, M. F., *J. Biol. Chem.*, **188**, 847-63 (1951)
28. McManus, I. R., *J. Biol. Chem.*, **188**, 729-40 (1951)
29. Plaut, G. W. E., and Lardy, H. A., *J. Biol. Chem.*, **180**, 13-27 (1949)
30. Herbert, D., *Symposia Soc. Exptl. Biol.*, **5**, 52-71 (1951)
31. Utter, M. F., and Wood, H. G., *Advances in Enzymol.*, **12**, 41-151 (1951)
32. Lichstein, H. C., *Vitamins & Hormones*, **9**, 27-74 (1951)
33. Torda, C., and Wolff, H. G., *J. Pharmacol. Exptl. Therap.*, **98**, 358-65 (1950)
34. Byerrum, R. U., Brown, S. A., and Ball, C. D., *Arch. Biochem.*, **26**, 442-56 (1950)
35. Nossal, P. M., *Australian J. Exptl. Biol. Med. Sci.*, **27**, 321-29 (1949)
36. Grafflin, A. L., and Ochoa, S., *Biochim. et Biophys. Acta.*, **4**, 205-10 (1950)

37. Whatley, F. R., *New Phytologist*, **50**, 258-67 (1951)
38. Kornberg, A., and Pricer, W. E., Jr., *J. Biol. Chem.*, **189**, 123-36 (1951)
39. Hewitt, E. J., *Ann. Rev. Plant Physiol.*, **2**, 29-31 (1951)
40. Speck, J. F., *J. Biol. Chem.*, **178**, 315-24 (1949)
41. Kornberg, A., Ochoa, S., and Mehler, A. H., *J. Biol. Chem.*, **174**, 159-72 (1948)
42. Kornberg, A., *Proc. 6th Intern. Congr. Exptl. Cytol.*, 1947, *Exptl. Cell Research Suppl.* **1**, 277-80 (Stockholm, Sweden, 1949)
43. Nossal, P. M., *Australian J. Exptl. Biol. Med. Sci.*, **27**, 313-20 (1949)
44. Steinberger, R., and Westheimer, F. H., *J. Am. Chem. Soc.*, **71**, 4158 (1949)
45. Steinberger, R., and Westheimer, F. H., *J. Am. Chem. Soc.*, **73**, 429-35 (1951)
46. Lwoff, A., *Proc. 6th Intern. Congr. Exptl. Cytol.*, 1947, *Exptl. Cell Research Suppl.* **1**, 290-91 (Stockholm, Sweden, 1949)
47. Lwoff, A., and Ionesco, H., *Ann. inst. Pasteur*, **74**, 442-50 (1948); **79**, 14-19 (1950)
48. Nossal, P. M., *Biochem. J.*, **49**, 407-13 (1951)
49. Korkes, S., del Campillo, A., and Ochoa, S., *J. Biol. Chem.*, **187**, 891-905 (1950)
50. Kaufman, S., Korkes, S., and del Campillo, A., *J. Biol. Chem.*, **192**, 301-12 (1951)
51. Ochoa, S., Veiga Salles, J. B., and Ortiz, P. J., *J. Biol. Chem.*, **187**, 863-74 (1950)
52. Nossal, P. M., *Biochem. J.*, **50**, 349-55 (1952)
53. Vishniac, W., and Ochoa, S., *Nature*, **167**, 768-69 (1951)
54. Ochoa, S., and Vishniac, W., *Organic Acid Metabolism in Higher Plants* (Godard, D. R., Ed., Iowa State College Press, Ames, Iowa, in press, 1952)
55. Tolmach, L. J., *Nature*, **167**, 946-48 (1951)
56. Tolmach, L. J., *Arch. Biochem. Biophys.*, **33**, 120-42 (1951)
57. Arnon, D. I., *Nature*, **167**, 1008-10 (1951)
58. Whatley, F. R., *New Phytologist*, **50**, 244-57 (1951)
59. Anderson, D. G., Paper presented before Am. Soc. Plant Physiol. (Minneapolis, Minn., Sept., 1951)
60. Tewfik, S., and Stumpf, P. K., *J. Biol. Chem.*, **192**, 519-26 (1951)
61. Thomas, M., *Symposia Soc. Exptl. Biol.*, **5**, 72-93 (1951)
62. Wolf, J., *Planta*, **37**, 510-34 (1949)
63. Gibbs, M., *Plant Physiol.*, **26**, 549-56 (1951)
64. Stutz, R. E., and Burris, R. H., *Plant Physiol.*, **26**, 226-43 (1951)
65. Calvin, M., Bassham, J. A., Benson, A. A., Lynch, V. H., Ouellet, C., Schou, L., Stepka, W., and Tolbert, N. E., *Symposia Soc. Exptl. Biol.*, **5**, 284-305 (1951)
66. Gaffron, H., Fager, E. W., and Rosenberg, J. L., *Symposia Soc. Exptl. Biol.*, **5**, 262-83 (1951)
67. Larsen, H., *J. Biol. Chem.*, **193**, 167-73 (1951)
- 67a. Barban, S., and Ajl, S. J., *J. Biol. Chem.*, **192**, 63-72 (1951)
68. Johns, A. T., *Nature*, **164**, 620-21 (1949)
69. Delwiche, E. A., *J. Bact.*, **59**, 439-42 (1950)
70. Lorber, V., Lifson, N., Sakami, W., and Wood, H. G., *J. Biol. Chem.*, **183**, 531-38 (1950)
71. Singer, T. P., and Pensky, J., *Arch. Biochem. Biophys.*, **31**, 457-59 (1951)
72. Watt, D., and Krampitz, L. O., *Federation Proc.*, **6**, 301 (1947); Juni, E., *Federation Proc.*, **9**, 396 (1950)
73. Dolin, M. I., and Gunsalus, I. C., *J. Bact.*, **62**, 199-214 (1941)
74. Ochoa, S., *Physiol. Revs.*, **31**, 56-106 (1951)

75. Vennesland, B., *The Enzymes*, **2**, Part 1, 183-215 (Sumner, J. B., and Myrback, K., Eds., Academic Press, Inc., New York, N. Y., 1951)
76. Reed, L. J., DeBusk, B. G., Gunsalus, I. C., and Hornberger, C. S., Jr., *Science*, **114**, 93-94 (1951)
77. Reed, L. J., DeBusk, B. G., Gunsalus, I. C., and Schnakenberg, G. H. F., *J. Am. Chem. Soc.*, **73**, 5920 (1951)
78. Stern, J. R., and Ochoa, S., *J. Biol. Chem.*, **191**, 161-72 (1951)
79. Ochoa, S., Stern, J. R., and Schneider, M. C., *J. Biol. Chem.*, **193**, 691-702 (1951)
80. Stern, J. R., Shapiro, B., Stadtman, E. R., and Ochoa, S., *J. Biol. Chem.*, **193**, 703-20 (1951)
81. Korkes, S., del Campillo, A., Gunsalus, I. C., and Ochoa, S., *J. Biol. Chem.*, **193**, 721-35 (1951)
82. Fager, E. W., *Arch. Biochem. Biophys.* (In press)
83. Aronoff, S., *Arch. Biochem. Biophys.*, **32**, 237-48 (1951)
84. Ajl, S. J., *Bact. Rev.*, **15**, 211-44 (1951)
85. Lewis, K. F., and Weinhouse, S., *J. Am. Chem. Soc.*, **73**, 2500-3 (1951)
86. Lewis, K. F., and Weinhouse, S., *J. Am. Chem. Soc.*, **73**, 2906-9 (1951)
87. Benson, A. A., Bassham, J. A., and Calvin, M., *J. Am. Chem. Soc.*, **73**, 2970 (1951)
88. Benson, A. A., *J. Am. Chem. Soc.*, **73**, 2971-72 (1951)
89. Bassham, J. A., Benson, A. A., and Calvin, M., *Program Am. Soc. Plant Physiol.*, **17** (Minneapolis, Minn., Sept. 9-12, 1951)
90. Bennet-Clark, T. A., *New Phytologist*, **32**, 128-61 (1933)
91. Bonner, J., *Plant Biochemistry*, 156 (Academic Press, Inc., New York, N. Y., 537 pp., 1950)
92. Hudson, C. S., *Advances in Carbohydrate Chem.*, **1**, 1-36 (1945)
93. Richtmyer, N. K., *Advances in Carbohydrate Chem.*, **1**, 37-76 (1945)
94. Pratt, J. W., Richtmyer, N. K., and Hudson, C. S., *J. Am. Chem. Soc.*, **73**, 1876-77 (1951)
95. Klevstrand, R., and Nordal, A., *Acta Chem. Scand.*, **4**, 1320 (1950)
96. Nordal, A., and Klevstrand, R., *Acta Chem. Scand.*, **5**, 85-88, 898-900 (1951)
97. Bevenue, A., and Williams, K. T., *Arch. Biochem. Biophys.*, **34**, 225-27 (1951)
98. Horecker, B. L., Racker, E., Cohen, S. S., Lipmann, F., Lampen, J. O., Schlenk, F., and Dische, Z., *A Symposium of Phosphorus Metabolism*, 117-203 (McElroy, W. D., and Glass, B., Eds., Johns Hopkins Press, Baltimore, Md., 1951)
99. Marmur, J., and Schlenk, F., *Arch. Biochem. Biophys.*, **31**, 154-55 (1951)
100. Schou, L., Benson, A. A., Bassham, J. A., and Calvin, M., *Physiol. Plantarum*, **3**, 487-95 (1950)
101. Clagett, C. O., and Tolbert, N. E., and Burris, R. H., *J. Biol. Chem.*, **178**, 977-87 (1949)
102. Tolbert, N. E., Clagett, C. O., and Burris, R. H., *J. Biol. Chem.*, **181**, 905-14 (1949)
103. Tolbert, N. E., and Burris, R. H., *J. Biol. Chem.*, **186**, 791-804 (1950)
104. Örström, A., *Arch. Biochem. Biophys.*, **33**, 484-85 (1951)
105. Wood, H. G., *Ciba Foundation Conf. Isotopes Biochem.* (Wolstenholme, G. E. W., Ed., J. & A. Churchill Ltd., London, England, 1951)
106. Matthews, M. B., and Vennesland, B., *J. Biol. Chem.*, **186**, 667-82 (1950)
107. Davison, D. C., *Biochem. J.*, **49**, 520-26 (1951)
108. Conn, E. E., and Vennesland, B., *J. Biol. Chem.*, **192**, 17-28 (1951)

109. Cori, O., and Lipmann, F., *J. Biol. Chem.*, **194**, 417-25 (1952)
110. Isbell, H. S., and Hudson, C. S., *Bur. Standards, J. Research*, **8**, 327-38 (1932)
111. Bentley, R., and Neuberger, A., *Biochem. J.*, **45**, 584-90 (1949)
112. Strecker, H. J., and Korkes, S., *Nature*, **168**, 913-14 (1951)
113. Horecker, B. L., and Smyrniotis, P. Z., *Arch. Biochem.*, **29**, 232-33 (1950)
114. Horecker, B. L., and Smyrniotis, P. Z., *J. Biol. Chem.*, **193**, 371-81 (1951)
115. Horecker, B. L., Smyrniotis, P. Z., and Seegmiller, J. E., *J. Biol. Chem.*, **193**, 383-96 (1951)
116. Cohen, S. S., and McNair Scott, D. B., *Science*, **111**, 543-44 (1950)
117. Cohen, S. S., and McNair Scott, D. B., *Nature*, **166**, 781-82 (1950)
118. McNair Scott, D. B., and Cohen, S. S., *J. Biol. Chem.*, **188**, 509-30 (1951)
119. Cohen, S. S., *J. Biol. Chem.*, **189**, 617-28 (1951)
120. McNair Scott, D. B., and Cohen, S. S., *J. Cellular Comp. Physiol.*, **38**, Suppl. 1, 173-201 (1951)
121. Dickens, F., and Glock, G. E., *Nature*, **166**, 33 (1950)
122. Dickens, F., and Glock, G. E., *Biochem. J.*, **50**, 81-95 (1951)
123. Schales, O., *The Enzymes*, **2**, part 1, 216-47 (Sumner, J. B., and Myrback, K., Eds., Academic Press, Inc., New York, N. Y., 1951)
124. Okunuki, K., *Botan. Mag. (Tokyo)*, **51**, 270-78 (1937)
125. Okunuki, K., *Acta Phytochimica (Japan)*, **13**, 155-59 (1943)
126. Schales, O., Mims, V., and Schales, S. S., *Arch. Biochem.*, **10**, 455-65 (1946)
127. Schales, O., and Schales, S. S., *Arch. Biochem.*, **11**, 155-66 (1946)
128. Schales, O., and Schales, S. S., *Arch. Biochem.*, **11**, 445-50 (1946)
129. Werle, E., and Bruninghaus, S., *Biochem. Z.*, **321**, 492-99 (1951)
130. Hasse, K., and Schumacher, H. W., *Chem. Ber.*, **83**, 68-71 (1950)
131. Beevers, H., *Biochem. J.*, **48**, 132-37 (1951)
132. Steward, F. C., Thompson, J. F., and Dent, C. E., *Science*, **110**, 439-40 (1949)
133. Steward, F. C., and Thompson, J. F., *Ann. Rev. Plant Physiol.*, **1**, 233-64 (1950)
134. Steward, F. C., Thompson, J. F., Millar, F. K., Thomas, M. D., and Hendricks, R. H., *Plant Physiol.*, **26**, 123-35 (1951)
135. Westall, R. G., *Nature*, **165**, 717-18 (1950)
136. Hulme, A. C., and Arthington, W., *Nature*, **165**, 716-17 (1950)
137. Hanke, M. E., and Siddiqi, M. S. H., *Federation Proc.*, **9**, 181 (1950)
138. Koppelman, R., Mandeles, S., and Hanke, M. E., *Federation Proc.*, **11**, 242 (1952)
139. Shemin, D., and Wittenberg, J., *J. Biol. Chem.*, **192**, 315-34 (1951)
140. Granick, S., *Ann. Rev. Plant Physiol.*, **2**, 115-44 (1951)
141. Werle, E., and Raub, A., *Biochem. Z.*, **318**, 538-53 (1948)
142. Buchanan, J. M., *J. Cellular Comp. Physiol.*, **38**, Suppl. 1, 143-171 (1951)
143. *Brookhaven Conf. Rept.*, *BNL 70 (C-13)* (1950)
144. *Symposia Soc. Exptl. Biol.*, **5** (Academic Press, Inc., New York, N. Y., 342+vii pp., 1951)
145. Ochoa, S., *The Enzymes*, **2**, part 2, 929-1032 (Sumner, J. B., and Myrback, K., Eds., Academic Press, Inc., New York, N. Y., 1951)

TREE PHYSIOLOGY¹

BY BRUNO HUBER

Institute of Forest Botany, University of Munich, Munich, Germany

DEFINITION

Literally understood, the physiology of trees comprises all physiological investigations on trees. The question is, however, whether or not a classification of physiology according to the type of plant is of any use. It is unlikely that one would write on the physiology of shrubs, herbs, Amarillidaceae, etc. Nevertheless, it has become a habit to consider the physiology of trees a special branch of the the physiology of plants. This fact is justified, first of all, by the special research methods required because of the dimensions of these largest objects of the plant kingdom. Generally, instead of the methods of the laboratory there must be used those of experimental ecology for open air conditions. He who works only with tree seedlings because he does not like to adapt his methods to the size of the trees is not truly a student of the physiology of trees.

In the strictest sense, the physiology of trees is a special branch of knowledge only if it is concerned with the study of those facts related to the dimensions of a tree or a forest, e.g., the correlation of growth with long shoots and short shoots and with sun leaves and shade leaves, the impressive performances of sap flow, including the daily phase-displacement made evident by the long lines of transport, the especially distinct periodical phenomena inherent in the longevity of trees, including the multi-year periods of fruit-bearing, and the peculiarities of forest plant breeding.

Therefore, I do not consider it the task of this report to elaborate on all the physiological research on trees, even of the last few years. Rather, I wish to illustrate the characteristic peculiarities of the tree-physiology point of view with three examples taken from the physiology of nutrition, of growth, and of reproduction. Temporally, I am continuing my lecture given in 1937 at the annual meeting of the German Botanical Society (1) which is, as far as I know, the last review of the branch of physiology treated herein.

PHYSIOLOGY OF METABOLISM

Carbon assimilation, transpiration, and respiration.—The gaseous exchange of entire small plants can be examined; in contrast, only that of small branches can be measured when the plant is a tree. Their respiration and assimilation can be estimated with conventional modern methods *in situ* by enclosing the branch in a glass container during the experiment. [Because of the unnatural climate in the glass container, these experiments

¹ The survey of the literature pertaining to this review was concluded in July, 1951.

should not last too long, i.e., about 3 min. By repeated measurements at intervals of 1 to 3 hr. one can get sufficient records of the daily march of photosynthesis of an individual branch (2).] In the determination of transpiration, such direct experiments are not as common (3, 4) as the much simpler weighing of excised branches.² The differences in weight resulting from transpiration can be determined in a few minutes on a sensitive torsion balance; thus, the effect of differences because of changes of stomatal widths is negligible.

As a result of these experiments, the rank of the main woody species with respect to the intensity of their gaseous exchange is established fairly well (2, 6, 7), as illustrated in Table I.

TABLE I
MEAN DAILY TRANSPIRATION (T), RESPIRATION (R), AND GROSS ASSIMILATION (A+R), ACCORDING TO POLSTER (2)

	T	R	A+R
<i>Betula verrucosa</i>	9500*	22.9*	66.9*
<i>Quercus robur</i>	6020	17.5	43.2
<i>Fagus sylvatica</i>	4830	11.1	52.9
<i>Larix decidua</i>	3340	7.9	27.0
<i>Pinus silvestris</i>	1880	7.9	17.1
<i>Pseudotsuga taxifolia</i>	1330	7.5	18.8
<i>Picea abies</i>	1380	5.5	14.2

* Milligrams per gram fresh weight.

The light-loving birch transpires, respire, and assimilates most actively; least active are the shade-loving trees, spruce and Douglas fir. Although the three kinds of gaseous exchange are rather similar because of the stomatal regulation, their proportions show certain differences. To produce 1 mg. of dry matter, the light-loving trees usually transpire and respire more than the shade-loving trees. This is especially true for the oak as compared to the beech, the latter assimilating more, but transpiring and respiring less than the oak.

The figures of Table I are based on young trees. Similar measurements for large trees are more difficult to obtain because of the great differences in carbon assimilation by the different parts of the crown. These have been investigated, as yet, only to a small extent. Pisek & Tranquilini (8) examined the transpiration of a high spruce during an entire vegetative season including a period of prolonged drought. The transpiration of the lower branches is slightly higher than that of the upper branches when the water supply

² Oppenheimer (5) writes on the history of this method. It was first used by Pfaff as early as 1870.

is good. Under drought conditions, the more sensitive shaded branches stop transpiring first, so that the relation is reversed. The comparative rates of assimilation of sun branches and shade branches are still essentially as classically stated by Boysen-Jensen (9); however, Pisek & Tranquilini have new experiments in progress on assimilation in the crowns of trees.

The latest efforts try to determine the gaseous exchange of entire plant communities. For this purpose, meteorology furnishes two methods. According to Schmidt (10), the vertical gas flow over a sufficiently extended area of vegetation can be determined as the product of the gradient of chemical composition multiplied by the "Austausch" [the coefficient of eddy motion (11 to 15)]. Because of transpiration, for instance, the absolute

TABLE II
DAILY MARCH OF CO₂ CONSUMPTION*
(APRIL 25 TO 26, 1950)

Time (hours)	CO ₂ consumption (arbitrary units)
8 P.M.-5 A.M.	-4.3
6 A.M.	-0.1
7 A.M.	5.0
8 A.M.-10 A.M.	16.0
10 A.M.-12 M.	16.4
1 P.M.	8.7
2 P.M.	2.8
6 P.M.	5.7
7 P.M.	1.5
8 P.M.	-3.9

* According to Huber (15).

humidity of the open air increases by about 10 per cent above the vegetation; because of assimilation, the CO₂ content decreases during the day by about 0.5 per cent. By important and ingenious instrumental improvements, the humidity as well as the CO₂ content at different heights above soil level can be measured with a high degree of accuracy.³ The gradient alone is not a sufficient measure for the intensity of vertical gas flow. The greater the turbulence of the air, the smaller is the gradient for the same gas flow. Therefore, only by multiplication of the gradient by a coefficient for turbulence can one obtain correct figures. In this manner, for instance, the daily and yearly march of assimilation and transpiration over a grass vegetation was determined, as shown in Tables II and III.

³ In this connection, the dew-point recorder (13) and the infrared-absorption recorder for CO₂ determination (15, 16, 17) should be mentioned.

TABLE III

YEARLY PERIODICITY OF RAINFALL AND EVAPORATION*

Time (months)	Precipitation (inches)	Evaporation (inches)
January	3.41	0.48
February	incomplete record	
March	2.81	0.92
April	2.93	1.07
May	0.20	1.65
June	5.87	2.44
July	2.16	1.93
August	2.98	1.46
September	incomplete record	
October	2.38	0.79
November	1.40	0.59
December	2.20	0.55
Total (10 months)	26.34	11.88

* According to Thornwaite & Holzman (11, 12).

Since evaporation constitutes about 30 per cent of the energy turnover of the earth, the evaporation (E) can be determined by the thermal balance equation of Albrecht (18). If the total radiation (R), as well as the heat conduction in soil (S) and air (A), can be measured fairly well, then

$$R = S + A + E \quad \text{or} \quad E = R - (S + A)$$

Berger-Landefeldt (19) compared this method of thermal balance for estimating evaporation with the conventional weighing method for the determination of transpiration and found them in good agreement with respect to the daily march.

Geurten solved an important special problem by detailed quantitative tests on the transpiration and the respiration of bark (4). It is surprising that the daily march of transpiration from bark does not follow that of evaporation, but decreases from about 10 o'clock on as a result of incipient drying. Respiration of bark is relatively much faster than that of leaves and reflects the much steeper CO_2 gradient which exists between the composition of gas in the wood and in the open air according to the experiments of MacDougal and co-workers (20, 21). Transpiration and respiration were determined *in situ* by absorption with silicagel and sodium hydroxide, respectively.

Sap flows.—An important problem in tree physiology is that of the sap flow. The large volumes transported over long distances in trees accentuate

phenomena which are negligible in small plants. Especially evident is the phase-displacement which became known through studies on sap flow and the movement of food materials. The speed of sap flow in different parts of the crown is not uniform; rather, the flow begins in the morning in the tips and continues gradually to the roots. Conversely, the flow still continues in the lower parts when it has already stopped in the branches (22, 23). The differences in flow are compensated by the water reserves in the trunk; as a symptom of these differences, the fluctuations in circumference of tree trunks were studied in detail by MacDougal (24).

Gibbs (25) has recently studied the seasonal changes in the water content of trees. Broad-leaved trees exhibiting root pressure, like *Betula*, show their greatest water content just before leaf-opening. A drying of the wood takes place during the summer months followed by a slight recovery of water content during defoliation and a gradual decrease as the winter season progresses, up to the time of refilling by root pressure when the ice is breaking. Ring-porous trees always have dry heartwood and only add a new ring of sapwood rich in water each spring. The sapwood of softwooded trees shows slight seasonal variations in moisture. Barks show the opposite behavior inasmuch as during the climax of growth (June and July) the highest water contents are often reached (sap-period). Such information is also important for the practice of floating.

Similar facts are known about the movement of food materials. The light-dependency of photosynthesis causes a large increase in the concentration of sugar in the morning which flows down the sieve tubes like a wave, reaching the base of the crown of small red oaks about 10 o'clock in the morning, of taller red oaks about 1 o'clock in the afternoon, and the base of the trunk at 7 o'clock in the evening (26, 27). This concentration wave is even reflected in the production of honey dew by plant lice which suck the sap of the sieve tubes (28, 29).

Whereas the thermoelectric method informed us in detail about the velocity of sap flow in conifers and in diffuse-porous and ring-porous, broad-leaved trees as well as the distribution of velocities within a tree (22, 23, 30), similar experiments on the velocity of the movement of assimilates are still lacking, although they should be possible with the fluorescein method (31, 32). In accordance with anatomical considerations (33, 34), similar differences in the velocity of movement of assimilates are to be expected between conifers (with sieve cells only) and broad-leaved trees (with narrow-porous, scalariform or broad-porous, simple sieve plates).

The chemical composition of the content of vessels and of sieve tubes of trees has also been examined. Bennett, Anderssen & Milad (35) developed methods for the extraction of the content of vessels. The content can be displaced by compressed air and the formation of menisci avoided by forcing in water. The opinion is confirmed that the content of vessels is a solution of electrolytes similar in composition to the soil-solution; a quantitative

comparison, however, is still lacking. Particularly important is the discovery that the concentration of the content of vessels decreases as the velocity of sap flow increases (36). It has not yet been decided whether the concentration of the content of vessels is inversely proportional to the speed of the flow, i.e., if the absorption of inorganic substances is completely independent of transpiration, as Hoagland (37) seems to presume.

The content of the sieve tubes, which exudes upon cutting, is 100 to 1000 times more concentrated than that of the vessels. It contains 10 to 30 per cent dry substance (mainly sugars, little nitrogenous compounds, auxins during the growing season, quite a number of enzymes, and, according to spectroanalysis, the elements boron, manganese, zinc, etc., in traces) a very complete nutrient solution. In deciduous trees, catabolic products from leaf protein breakdown, namely, increasing quantities of nitrogen and hydrogen sulphide, appear before defoliation. It is known that viruses, flagellates, and other causes of disease can also be transported by sieve tubes.⁴

The concentration of the sap of sieve tubes decreases from the assimilating tissue of the leaf downward. There appears to be an active secretory mechanism involved since according to Röckl (41) the osmotic concentration increases from the palisade tissue of the leaf to the sieve tubes by way of the collecting cells and the bundle sheath with the result that the palisade tissue can be plasmolysed in the sap of the sieve tubes.

PHYSIOLOGY OF GROWTH AND DEVELOPMENT

The laws governing the growth of trees over extended periods of time are of high practical importance. For this reason they have long been the subject of a special aspect of forestry called "Growth and Yield Research." The results are compiled in several manuals, the most recent being *Einführung in die forstliche Zuwachs- und Ertragslehre* written by Vanselow (42). There is a regular increase and decrease in the vigor of extension as well as radial growth, and therefore of growth in volume (the "Grand Period" of Sachs). A mathematical expression for this growth behavior has been sought by many authors, most recently by Peschel (43) and Weck (44). The most important theoretical progress of the last 10 years is, in my opinion, the discovery made by Backman of Sweden (45) who stated that the unsymmetrical growth curves become somewhat symmetrical when time is plotted logarithmically instead of linearly, so that the long time of decreasing growth is contracted. Also, the natural age limit of trees can be extrapolated by this method [Molisch (46)].

The attempt to study the daily course of radial growth by measurement of the circumference of trees was, as is well known, not fully successful,

⁴ Compare the reviews on phloem exudation by Huber (38) and by Crafts (39); also see Tammes (40).

because the effects of the diurnal variation in water content on the circumference are more extensive than those caused by the growth proper.⁵ Friedrich (47) undertook these measurements with his "growth autograph" as early as 1897 and MacDougal devoted an important part of his lifetime to this problem [summarized in (48)].

The main effort of the research of the past few years on the growth of trees has therefore been devoted to the seasonal variation in growth, especially the radial growth over the length of the tree shaft.⁶ Coster, Priestley, and many others (50 to 54) confirmed and extended the old idea that the radial growth generally progresses basipetally with the flow of hormones and assimilates from the crown downward. In soft and in diffuse-porous hardwoods, it may take several weeks before cambial activity, beginning in the crown, reaches the base of the trunk, whereas in ring-porous trees the activity is found over the whole trunk in one or a few days. The exact knowledge of these phenomena found practical application in the procurement of tannin bark. It was considerably advanced in Germany during the war (55, 56). A clear relation exists between auxin concentration and the ease of peeling of barks. When auxins are prevented from moving by girdling, it is possible to peel the bark for several more weeks during the autumn.

In addition to the easily recognizable initiation of growth in the spring, one of the most interesting questions is whether the growth increases rather linearly, then gradually decreases, and eventually fluctuates with the conditions of the surroundings (temperature, rainfall), or if there is a division of the vegetative season by a certain "midsummer-rest," like the depression at noon in the daily march of gaseous exchange. Several arguments could be found for this widespread belief in a "second sap period" (55). For instance, in *Abies alba* "double rings" are very often found because after the beginning of the formation of late wood, another increase in growth—without being clearly outlined—brings forth some sort of early wood before the tree ring is finally completed.⁷

Important objective methods have lately been developed for distinguishing between early and late wood, such as the photometric method of Müller-Stoll (58), the "Härtetaster" (instrument for measuring the degree of hardness) of Mayer-Wegelin (59), and the sulphuric acid corrosion test of Kisser & Lehnert [(60); see also (61)].

⁵ An improved dendrographic method has recently been described by Byram & Doolittle (49).

⁶ These measurements of radial growth are based mainly on the microscopic examination of boring cores and strips as prepared in accordance with Priestley's method (51, 52).

⁷ The double period of vegetation, reported by Engler in Switzerland, in relation to the extension growth of roots of spruce and beech, could not be confirmed by Ladefoged (57) for moist Denmark. There, the root growth of both species culminates in one peak in July.

The longevity of trees, however, demands the study of still longer periods of growth and development. In the vegetative life of a tree there are, in addition to the fluctuations in the width of tree rings, periodicities in the formation of periderm and the so-called "Absprünge" (cladoptosis, the spontaneous shedding of twigs); in the reproductive life there is the rhythm of the seed years.

Holdheide microscopically observed tree rings in most of the central European barks and determined that only some of the trees, like *Vitis*, *Clematis*, and *Populus*, lose one annual ring with each abstricted fragment of periderm; each fragment of periderm from most of the trees contains several rings (up to 30 in *Acer pseudoplatanus*), so that years may pass before a new phellogen is formed at a particular point. It is, however, undecided whether certain years favor the formation of new phellogens all over the trunk, or whether such phellogens appear sporadically each year so that several years pass before a particular spot has its turn again.

Experiments on the release of "Absprünge" are lacking. We have to thank Gäumann, however, for a minute chemical analysis of the metabolism of a beech forest which, as is well known, produces seed only after long years of storing up reserve materials. About three-quarters of these reserve materials are consumed by the seed production (63). The seed years, therefore, mark themselves by a minimum in the width of the tree rings (64).

Research on tree rings has resulted in the accumulation of a great deal of data in the field of long-term growth variations, recorded especially in the 17 volumes of the *Tree Ring Bulletin*. Following the methods of the American school [Douglass (65); Schulman (66)], extensive data on tree ring periodicities have been compiled lately and evaluated with respect to climate in different countries of Europe [Scandinavia (67, 68); Germany (69, 70, 71); and Italy (72)], as well as in Anatolia (73) and Southwest Africa (74). The studies show that in the southern drought areas—as early observed by Leonardo da Vinci [cited by Buli (72)]—the width of the tree rings more or less follows fluctuations in rainfall; in the North—mentioned for the first time by Linné (75)—those of temperature. It is therefore necessary to drop the optimistic expectation of De Geer (76) who held that the variations in the width of tree rings result from cosmic factors and have world-wide validity. An exact mathematical analysis by Müller-Stoll (77) of the geographical range of comparable variations in width shows that in central Europe there are surprisingly comparable variations over distances of more than 1000 km. so that the curves of tree ring widths for different areas can be synchronized; but such correlation is difficult between Scandinavia and Germany and even more so between Europe and North America.

The drought year of 1947 in Europe, the most severe in more than 100 years, provided an opportunity to examine the effects of drought on the growth of trees (78 to 81). The full drought did not appear until mid-summer, after extension growth and radial growth were almost completed; conse-

quently, it was the 1948 tree ring that was narrow. Recovery started in the upper parts of the tree earlier than in the base of the trunk. Spruce (*Picea abies*) in the artificial cultivation areas located below the natural mountainous habitats were most heavily injured. Many trees were killed immediately while others fell victim to secondary diseases like bark beetles and honey fungus (*Agaricus melleus*).

In addition to variations in growth over extended periods of time, very peculiar local differences in growth exist. Bailey calculated that the increase in circumference of conifers requires each cambial cell to produce, on the average, a sister cell once every 15 years. A detailed examination of series of tangential and radial sections by Bannan & Whalley (82, 83, 84) showed a rate of division about 100 times greater. It became obvious that not all of the cambial cells formed continue living but that most of them sooner or later stop functioning and become overgrown by adjacent cells. The radial growth of the trees studied, therefore, does not follow the laws of strict individual economy, but instead, those more applicable to competition within populations. The reviewer believes this to be a law of great biological importance and also believes that the old age of many trees is based on this inner rejuvenation.

In some cases the zones of more or less intensive growth are clearly defined anatomically. For instance, in the bark of *Prunus* the rays grow much less actively than the intervening fascicular tissue (62, 85, 86). With *Carpinus*, also, the aggregated rays make most striking shriveled zones in between dilated areas causing the surface of the bark to appear as a network of lighter and darker areas (62, 87). A multitude of unsolved problems exist in connection with the physiology of bark development and the many secondary alterations, such as the collapse of sieve tubes, the inflation of parenchyma cells, sclerosis, dilatation, and finally, the formation of cork.

PHYSIOLOGY OF REPRODUCTION

There is little doubt that the laws of heredity apply to trees just as to other organisms. Little is known, however, about tree genetics, and systematic tree breeding is of very recent origin because of the longevity and late fertility of trees. I will point out briefly at the end of this review the promising potentialities of tree breeding, a subject specifically discussed in 1950 by His Royal Highness, the Crown Prince of Sweden (the present king), on the occasion of the 7th International Botanical Congress held in Stockholm. The most modern survey of this subject is that by Lindquist (88) in *Genetics in Swedish Forestry Practice*, published in 1946 in Swedish, in 1948 in English, and in 1951 in German. On the whole, Sweden is leading in this branch of science. The basis for forest tree breeding in a reasonable period of time has been a good knowledge of vegetative reproduction as well as of seed production. The former has been greatly facilitated by use of synthetic growth hormones (89). In case cuttings will not root, grafting

them on a stock very often helps and is particularly successful with *Larix* and adequate with *Pinus* and *Picea*.

The grafting technique makes it unnecessary to depend on the scarcely obtainable seed from the top of trees;⁸ instead, twigs from the top of the trees capable of blossoming are simply grafted into young plants where they continue to blossom and to bear fruit. In addition to making seeds accessible, such "seed plantations" make possible all sorts of hybridizations and thus the study of inheritance (91). With the *Populus* and *Salix*, pollination and the production of seeds can even be carried out with cut branches placed in water.

The grafting technique, using shoots of fertile trees is preferable to selecting for early blossoming individuals. Earliness of ripening often makes demands on the vegetative growth potential. For instance, the "Alnus dying" is based, according to Münch (92) on unwise selection by seedmen of an early blossoming breed which is, unfortunately, also short-lived. With dioecious plants, the consumption of food materials for the production of seeds results in a considerably decreased production of timber by female specimens [according to Rohmeder (93), there is up to a 40 per cent reduction with the physiologically dioecious *Fraxinus excelsior*]. Therefore, forestry, which is concerned with timber production and not fruitfulness must beware of one-sided selection for fertility.

Carefully selected trees must be obtained before laying out a seed plantation. Since one birch or a few pines will suffice for the requirements of entire countries, a very high standard can be applied in this selection. At first the selection can only cover phenotypes, thus conclusive statements as to their real value in breeding can only be made when the analysis of the progeny becomes available. Today, only a few experimental forest tree breeding plantations are old enough to form a basis for judgement.⁹ Nevertheless, valuable information is available concerning the inheritance of straightness of shaft, fineness of branches, tortuous and gnarl growth, early and late leaf development, disease-resistance, etc. Judgement must be reserved because of the longevity of the trees on the most important character—the intensity of growth. A 20-year study of seedlings from individual trees of spruce by Münch (96) showed that the intensity of early growth is independent of the efficiency of later growth, i.e., individual seedlings,

⁸ In the 19th century, the collecting of cones was left to poor mountain farmers who, of course, gathered the seed mainly from low cripples. The results were disastrous. The catastrophe of the "dying of larches" (90), and the failure of the artificial growing of larches on thousands of acres resulted mainly from the effects of these unsuitable seeds. Today, each forestry office instructs its own cone-gathering parties, who gather the seed from acknowledged standard plantations.

⁹ Already, however, it is often possible to judge the inheritance of characteristics like bifurcation. The study of archives often gives clues as to the descent of elite stocks (94, 95) in districts that have been cultivated for a long time.

ranking first when 3 years old, could rank last when 20 years old and vice versa. It is peculiar that the German spruce, now cultivated in southern Sweden, yields much more timber than the native tree. This is explained by the fact that the spruce, by natural distribution, could have reached Sweden only by way of the Bothnic Gulf. Therefore, southern Sweden became colonized by a slowly growing northern spruce, although the rapidly growing German spruce, now artificially introduced, could have grown in the climate of Sweden.

Poplar breeding has had the greatest initial success because of the mass production of seed, the simplicity of rooting of cuttings, and the rapid growth (97). Here, the appearance of *heterosis*—the luxuriant growth of hybrids surpassing that of both parent trees—resulted in a surprising growth performance unknown with any other species in the temperate zones. Recently, similar results have been observed with *Larix* species (*L. europaea* × *L. leptolepis* and vice versa) and with some pines (98).

The forestry practice of thinning is a selection method used for centuries. The selection, until the establishment of the principle that poor trees fall first, has been mostly a counter-selection since, in thinning, the best trees were felled—the usual practice in all primitive countries. Experts in forest tree genetics expect that with a few decades of tree breeding, results similar to those attained with agricultural crop plants will be produced. The remarkable successes with poplar breeding permit such an expectation!

LITERATURE CITED

1. Huber, B., *Ber. deut. botan. Ges.*, **55**, 46 (1937)
2. Polster, H., *Die physiologischen Grundlagen der Stoffherzeugung im Walde* (Bayer. Landwirtschaftsverlag, München, Germany, 96 pp., 1950)
3. Schratz, E., *Jahrb. wiss. Botan.*, **74**, 153 (1931)
4. Geurten, I., *Forstwiss. Centr.*, **69**, 704 (1950)
5. Oppenheimer, H. R., and Mendel, K., *Palestine J. Botany. Rehovot Ser. 2*, 171 (1939)
6. Eidmann, F. E., *Schriftenreihe Akad. deutsch. Forstwiss.*, **5**, 1-144 (1943)
7. Pisek, A., and Cartellieri, E., *Jahrb. wiss. Botan.*, **88**, 22 (1939); **90**, 255 (1941)
8. Pisek, A., and Tranquillini, W., *Physiol. Plantarum*, **4**, 1 (1951)
9. Boysen-Jensen, P., *Die Stoffproduktion der Pflanzen* (G. Fischer-Verlag, Jena, Germany, 108 pp., 1932)
10. Schmidt, W., *Probleme kosmisch. Physik*, **7**, (1925)
11. Thornthwaite, C. W., and Holzman, B., *Trans. Am. Geophys. Union*, 510 (1940); 429 (1941)
12. Thornthwaite, C. W., and Holzman, B., *U. S. Dept. Agr. Tech. Bull. No. 817* (1942)
13. Thornthwaite, C. W., and Owen, J. C., *Monthly Weather Rev.*, **68**, 315 (1940)
14. Huber, B., *K. Akad. Wiss. Wien, Math.-naturw. Klasse I*, **155**, 97 (1947)
15. Huber, B., *Ber. deut. botan. Ges.*, **63**, 53 (1950)
16. Egle, K., and Ernst, A., *Z. Naturforsch.* **4b**, 351 (1949)
17. Strugger, S., and Baumeister, W., *Ber. deut. botan. Ges.*, **64**, 5 (1951)
18. Albrecht, F., *Reichsamt Wetterdienst, Wiss. Abhandl.*, **8/2** (1940)
19. Berger-Landefeldt, U., *Planta*, **37**, 6 (1949)
20. MacDougal, D. T., Overton, J. B., and Smith, G. M., *Carnegie Inst. Wash. Pub., No. 397* (1929)
21. MacDougal, D. T., and Working, E. B., *Carnegie Inst. Wash. Pub., No. 441* (1933)
22. Baumgartner, A., *Z. Botan.*, **28**, 81 (1934)
23. Huber, B., and Schmidt, E., *Tharand. forstl. Jahrb.*, **87**, 369 (1936)
24. MacDougal, D. T., *Carnegie Inst. Wash. Pub., No. 365* (1925)
25. Gibbs, R. D., *Abstract 7th Intern. Bot. Congr. (Stockholm, Sweden, 1950)*
26. Huber, B., Schmidt, E., and Jahnelt, H., *Tharand. forstl. Jahrb.*, **88**, 1017 (1937)
27. Huber, B., *Ber. deut. botan. Ges.*, **59**, 181 (1941)
28. Romell, L. G., *Svensk. Botan. Tid.*, **29**, 391 (1935)
29. Leonhardt, H., *Z. angew. Entomol.*, **27**, 208 (1940)
30. Schubert, A., *Tharand. forstl. Jahrb.*, **90**, 821 (1939)
31. Schumacher, A., *Planta*, **35**, 642 (1948)
32. Rouschal, E., *Flora*, **135**, 135 (1941)
33. Huber, B., *Jahrb. wiss. Botan.*, **88**, 176 (1939)
34. Esau, K., *Botan. Rev.*, **5**, 373 (1939); **16**, 67 (1950)
35. Bennett, J. P., Anderssen, F. G., and Milad, J., *New Phytologist*, **26**, 316 (1927)
36. Jahnelt, H., *Jahrb. wiss. Botan.*, **86**, 527 (1938)
37. Hoagland, D. R., *Lectures on the Inorganic Nutrition of Plants* (Chronica Botanica Co., Waltham, Massachusetts, 226 pp., 1944)
38. Huber, B., *Biol. Generalis*, **16**, 310 (1942)
39. Crafts, A. S., *Botan. Rev.*, **17**, 203 (1951)
40. Tammes, P. M. L., *Proc. Netherland Acad. Wetensch.*, [C]**54**, 3 (1951)

41. Röckl, B., *Planta*, **36**, 530 (1949)
42. Vanselow, K., *Einführung in die forstliche Zuwachs- und Ertragslehre*, 3rd ed. (Verlag Sauerlaender, Frankfurt, Germany, 156 pp., 1948)
43. Peschel, W., *Tharand. forstl. Jahrb.*, **89**, 169 (1938)
44. Weck, H., *Forstwiss. Centr.*, **69**, 12 (1950)
45. Backman, G., *Bios*, **15** (1943)
46. Molisch, H., *Die Lebensdauer der Pflanze* (G. Fischer-Verlag, Jena, Germany, 1929)
47. Friedrich, J., *Zentr. ges. Forstwesen*, **23**, 471 (1897)
48. MacDougal, D. T., *Tree Growth* (Chronica Botanica Co., Waltham, Massachusetts, 240 pp., 1938)
49. Byram, G. M., and Doolittle, W. T., *Ecology*, **31**, 27 (1950)
50. Coster, C., *Ann. Jardin. Botan. Buitenzorg*, **37**, 49 (1927); **38**, 1 (1927)
51. Priestley, J. H., *Forestry*, **9**, 84 (1935)
52. Priestley, J. H., Scott, L. I., and Malins, M. E., *Proc. Leeds Phil. Lit. Soc. Sci. Sect.*, **2**, 365 (1933); **3**, 42 (1935)
53. Chowdhury, K. A., and Tandan, K. N., *Nature*, **165**, 732 (1950)
54. Artschwager, E., *Am. J. Botany*, **37**, 15 (1950)
55. Huber, B., *Forstwiss. Centr.*, **67**, 129 (1948)
56. Huber, B., *Forstarchiv*, **20**, 10 (1944)
57. Ladefoged, K., *Det forstl. Forsøgsvesen i Danmark*, **16**, 1-256 (1939)
58. Müller-Stoll, W., *Planta*, **35**, 397 (1947)
59. Mayer-Wegelin, H., *Allgem. Forst- u. Jagd-Zt.*, **122**, 12 (1950)
60. Kisser, J., and Lehnert, I., *Intern. Holzmarkt, Mitt. österr. Ges. Holzforsch.*, **1**, 16 (1951)
61. Huber, B., in *Handbuch der Mikroskopie in der Technik*, **V/1**, 81 (Umschau-Verlag, Frankfurt, Germany, 1951)
62. Holdheide, W., in *Handbuch der Mikroskopie in der Technik*, **V/1**, 195 (Umschau-Verlag, Frankfurt, Germany, 1951)
63. Gäumann, E., *Ber. schweiz. botan. Ges.*, **44**, 157 (1935)
64. Jazewitsch, W. von, *Forstwiss. Centr.* (In press)
65. Douglass, A. E., *Carnegie Inst. Wash. Pub.*, No. 289 (1919)
66. Schulman, E., *Univ. of Arizona Bull.*, **16**(4), 1945; **18**(3) (1947)
67. Erlandsson, S., *Stockholms Högskolas Geokronolog. Inst.*, Data 23 (1936)
68. Ording, A., *Medd. Norske Skogforsøkvesen*, **7**, 105 (1941)
69. Huber, B., *Naturwissenschaften*, **35**, 151 (1948)
70. Huber, B., Jazewitsch, W. von, John, A., and Wellenhofer, W., *Forstwiss. Centr.*, **68**, 706 (1949)
71. Huber, B., and Jazewitsch, W. von, *Allgem. Forstzeit.*, **5**, 42, 49 (1950)
72. Buli, U., *Ricerche climatiche sulle pinete di Ravenna, Ricerche sulle variazioni storiche del clima Italiano*, **10** (Tipografia Maregiani, Bologna, Italy, 77 pp., 1949)
73. Gassner, G., and Christiansen-Weniger, F., *Nova Acta Leopoldina*, **12**(80), 137 (1942)
74. Walter, H., *Naturwissenschaften*, **28**, 607 (1940)
75. Linné, C. v., in Erlandsson, S., *Stockholms Högskolas Geokronolog. Inst.*, Data 23 (1936)
76. De Geer, E. H., *Stockholms Högskolas Geokronol. Inst.*, Data 22, 501 (1935)
77. Müller-Stoll, H., *Bibliotheca Botan.*, **122**, 93 (1951)
78. *Ber. des Deut. Wetterdienstes U. S. Zone*, No. 7 (1949)

79. Geiger, R., *Forstwiss. Centr.*, **70**, 349 (1951)
80. Schober, R., *Forstwiss. Centr.*, **70**, 204 (1951)
81. Kröner, H., *Vergleichende Untersuchungen über die Wirkungen des Dürresommers 1947 auf Langen- und Dickenwachstum unserer Bäume* (Doctoral thesis, Universität, München, Germany, 1951)
82. Bannan, M. W., *Am. J. Botany*, **37**, 511 (1950)
83. Bannan, M. W., and Whalley, B. E., *Can. J. Research*, [C]**28**, 341 (1950)
84. Whalley, B. E., *Can. J. Research*, [C]**28**, 331 (1950)
85. Schneider, H., *Bull. Torrey Bot. Club*, **72**, 137 (1945)
86. Schneider, H., *Phytopathology*, **35**, 610 (1945)
87. Holdheide, H. (Unpublished data)
88. Lindquist, B., *Genetics in Swedish Forestry Practice* (Svensk Skogsvesens-Foereningens Förlag, Stockholm, Sweden, 173 pp., 1948)
89. Avery, G. S., Jr., Johnson, E. B., Addoms, R. M., and Thompson, B. F., *Hormones and Horticulture, the Use of Special Chemicals in the Control of Plant Growth* (McGraw-Hill Book Co., New York, N. Y., 326 pp., 1947)
90. Münch, E., *Forstwiss. Centr.*, **58**, 641 (1936)
91. Wettstein, W. v., *Die Vermehrung und Kultur der Pappel*, 4th ed. (Sauerlaender-Verlag, Frankfurt, Germany, 49 pp., 1944)
92. Münch, E., *Forstwiss. Centr.*, **58**, 213 (1936)
93. Rohmeder, E., *Forstwiss. Centrbl.*, **68**, 680 (1949)
94. Langner, W., *Tharand. forstl. Jahrb.*, **86**, 1 (1935)
95. Paes, R., *Tharand. forstl. Jahrb.*, **86**, 281 (1935)
96. Münch, E., *Beiträge zur Forstpflanzenzüchtung* (Bayerischer Landwirtschaftsverlag, München, Germany, 118 pp., 1949)
97. Hesmer, H., *Das Pappelbuch* (Verlag des Deutschen Pappelvereins, Bonn, Germany, 340 pp., 1951)
98. Langner, W., *Z. Forstgenetik und Forstpflanzenzüchtung*, **1**, 2 (1951)

AUTHOR INDEX

A

Aberg, B., 70, 95
 Adams, W. L., 208, 214, 217, 218
 Addicott, F. T., 100
 Addoms, R. M., 88, 341
 Aginlan, A. A., 297
 Ahlgren, H. L., 88
 Ainsworth, G. C., 174
 Aji, S. J., 318, 320
 Albaum, H. G., 35-58, 37, 38, 39, 40, 41, 43, 44, 51, 53, 54
 Alberda, T., 127
 Albers, V. M., 254
 Alberty, R. A., 140
 Albrecht, F., 336
 Algera, L., 230
 Allard, H. A., 272, 274, 275, 276
 Allen, R. J. L., 43, 44
 Allen, T. C., 76, 90
 Allen, W. W., 87
 Altman, K. I., 310
 Ames, J. W., 207, 208, 214
 Andel, O. M. van, 115
 Anderson, B., 149
 Anderson, D. G., 315
 Anderssen, F. G., 337
 Andreae, W. A., 184
 Anson, M. L., 137, 245, 252
 Anthony, R. D., 212
 Arisz, W. H., 109-30, 109, 110, 111, 112, 113, 115, 118
 Ark, P. A., 14
 Arney, S. E., 36
 Arnold, W., 241, 245, 252, 253
 Arnon, D. I., 31, 210, 212, 216, 236, 237, 245, 247, 252, 260
 Aronoff, S., 137, 245, 246, 252, 320
 Arreguin-Lozano, B., 43, 44
 Arrington, L. G., 87
 Arthington, W., 327
 Artschwager, E., 339
 Asai, G. N., 118
 Asana, R. D., 126
 Asbury, C. E., 160
 Ashby, W. C., 61, 94
 Ashkinazi, M. S., 258, 259
 Ashton, F. M., 96
 Ashworth, J. N., 143
 Ashworth, R. de B., 89
 Atkinson, D., 61
 Audus, L. J., 79, 91, 96, 97

Auerbach, V. H., 27
 Avakian, A. A., 290, 295
 Avery, G. S., Jr., 76, 77, 79, 81, 88, 100, 341
 Avineri-Shapiro, S., 48
 Axelrod, B., 17, 24, 25, 26, 30, 41, 44, 51, 80, 81, 146, 308, 319

B

Baba, T., 27
 Backman, G., 338
 Baer, B. H., 157
 Bailey, J. M., 19, 21, 23
 Baker, G. L., 157
 Baker, R. S., 17, 26, 60, 90, 95
 Bakhuyzen, H. L. van de S., 294, 295, 299
 Bald, J. C., 173
 Ball, C. D., 91, 311
 Balls, A. K., 146
 Bandurski, R. S., 17, 24, 25, 26, 30, 41, 44, 51, 59-86, 80, 81, 308, 319
 Banfi, R. F., 311
 Bannan, M. W., 341
 Barban, S., 318
 Barbier, G., 5, 13
 Bard, R. C., 27
 Barkalis, S. S., 25
 Barker, H. A., 22, 24, 31
 Barker, J., 158
 Barker, J. W., 82
 Barker, S. A., 19, 20
 Bärlund, H., 112
 Barrentine, M., 212
 Barrien, B. S., 136
 Barrón, E. S. G., 25, 30, 309
 Bartholomew, R. P., 210, 214
 Bassham, J. A., 40, 49, 317, 321, 322
 Bastisse, E. M., 11
 Bates, P. K., 165
 Bate-Smith, E. C., 159
 Batjer, L. P., 100, 101
 Bauer, K. E., 212, 217, 220, 224
 Bauer, L., 111, 125
 Baumeister, W., 335
 Baumgartner, A., 337
 Baur, E., 174
 Bawden, F. C., 146, 171-88, 173, 174, 175, 176, 177, 178, 180, 181, 182, 183, 184
 Beacham, L. M., 158
 Beale, H. P., 173, 178, 179
 Beckmann, C. O., 21, 47
 Bedford, C. L., 149
 Bedford, R. H., 150, 164, 165
 Beeson, K. C., 212
 Beevers, H., 29, 94, 326
 Behnke, J., 103
 Behrens, G., 282, 288
 Behrens, M., 54
 Beijer, I. J., 230
 Bellinger, F., 153, 163
 Benedict, H. M., 273, 276, 281
 Bennet-Clark, T. A., 321
 Bennett, C. W., 123, 176, 180
 Bennett, H. P., 10
 Bennett, J. P., 337
 Bennett-Clark, J. A., 82
 Benson, A. A., 40, 47, 49, 307, 317, 321, 322
 Bentley, J. A., 61, 63, 94
 Bentley, R., 324
 Bevenue, A., 321
 Berger, E. P., 111
 Berger, J., 76, 77, 79
 Berger-Landefeldt, U., 336
 Bernfeld, P., 21, 22, 47
 Berry, J. A., 149, 158, 160, 164, 165
 Best, R. J., 175, 184
 Bever, A. T., 22, 23
 Bezinger, E. N., 235
 Bhargava, K. S., 174, 175, 179
 Biale, J. B., 150
 Biddulph, O., 116
 Biebel, J. P., 268, 269, 273, 275, 276, 281
 Bigelow, S. L., 165
 Biscoe, J., 134
 Bishop, J. R., 93
 Black, L. M., 171, 185
 Blackman, G. E., 78, 88, 95, 96
 Bledsoe, R. W., 122
 Blinks, L. R., 238
 Bliss, L., 22
 Blondeau, R., 66, 70, 94, 98, 99
 Bode, O., 276, 278, 282, 286, 298
 Boggs, M. M., 149, 158, 160, 161
 Bohart, C. S., 161
 Boichenko, E. A., 236, 251
 Bois, E., 48
 Boisshot, P., 5
 Boivin, A., 54
 Boken, E., 5, 6
 Bolas, B. D., 9
 Bondorff, K. A., 4

- Bonner, D., 90, 95, 287
 Bonner, J., 25, 30, 35, 39,
 42, 43, 44, 50, 51, 52,
 59-86, 59, 60, 61, 62, 65,
 66, 67, 69, 70, 71, 72, 73,
 74, 79, 80, 81, 88, 89,
 91, 92, 94, 95, 97, 100,
 131, 133, 134, 135, 139,
 141, 142, 143, 145, 146,
 147, 182, 183, 237, 269,
 270, 275, 276, 277, 278,
 279, 280, 282, 283, 287,
 297, 308, 319, 321
 Bonner, W. D., Jr., 50, 63,
 66, 67, 68, 70, 78, 89, 95
 Bonney, V. B., 149, 160
 Booi, H. L., 94
 Borg, R. J., 28
 Borthwick, H. A., 268, 270,
 273, 275, 276, 278, 281,
 282, 283, 295
 Boswell, J. G., 309
 Bot, G. M., 135, 136
 Both, M. P., 109, 123
 Bötticher, R., 115
 Bouillenne, R., 127
 Bourne, E. J., 17, 19, 20,
 23, 30, 47
 Bourne, M. C., 63
 Bourroughs, H., 78, 79
 Boyle, F. P., 60, 61, 94
 Boynton, D., 207, 212, 213,
 215, 216
 Boysen-Jensen, P., 335
 Bradfield, J. R. G., 236
 Brannaman, B. L., 99
 Brekke, J. F., 157
 Bremner, M., 7
 Brierley, P., 178, 179
 Briesse, K., 250
 Brin, G. P., 256
 Britton, H. T. S., 8
 Bromfield, S. M., 8
 Brooks, I. A., 252
 Brooks, S. C., 202
 Brouwer, R., 117
 Brown, A. H., 251
 Brown, E. H., 12
 Brown, H. D., 149
 Brown, J. G., 215, 216
 Brown, J. W., 94, 123
 Brown, R. J., 212, 213
 Brown, S. A., 311
 Brown, S. M., 207, 209,
 212, 213, 216, 217, 218,
 222
 Broyer, T. C., 115
 Bruninghaus, S., 326
 Bryan, J. H. D., 54
 Buchanan, J. M., 328
 Buck, R. E., 157
 Bul, U., 340
 Buller, A. H. R., 110
 Bullock, R. M., 101
 Bünning, E., 273, 286, 295
 Bunting, A. H., 17, 26
 Burk, D., 66
 Burkhart, L. F., 207
 Burr, G. O., 96, 110, 236
 Burris, R. H., 60, 76, 90,
 94, 116, 175, 309, 316,
 317, 322
 Burström, H., 68, 69, 70,
 78, 79, 116
 Burvill, G. H., 3
 Buslova, E. D., 268
 Byerrum, R. U., 311
 Byram, G. M., 339
- C
- Çallahian, M. H., 268, 270,
 273, 275, 282, 283, 287
 Cain, J. C., 213, 215, 216
 Caldwell, J. S., 159, 160
 Calvin, M., 40, 47, 49, 307,
 317, 321, 322
 Campbell, H., 149, 156, 158,
 161
 Campbell, H. T., 160
 Campbell, J., 134, 139, 142,
 143, 145
 Campbell, J. M., 39, 141,
 142, 143, 145, 281
 Campillo, A. del, 314, 319
 Camus, G. C., 141, 146
 Cannon, H. B., 212, 213,
 220
 Caplin, S. M., 102
 Cardini, C. C., 24, 31
 Carlisle, H., 88
 Carlson, R. F., 95
 Carlton, H., 149
 Carolus, R. L., 209, 213,
 214
 Carpenter, P. N., 212, 213,
 214
 Carroll, D., 3
 Carsner, E., 292, 297
 Cartellieri, E., 334
 Carter, C. E., 41
 Carter, N., 117
 Carter, T. F., 162
 Caul, J. F., 157
 Chabannes, J., 5, 13
 Chakravarti, S. C., 292
 Chandler, R. F., Jr., 207,
 213, 214, 220
 Chandler, W. H., 152
 Chang, C. W., 220
 Chang, H. T., 292
 Chapman, H. D., 10, 207,
 209, 212, 213, 214, 216,
 217, 218, 222, 224
 Chargaiff, E., 54
 Chaudhuri, R. P., 184
 Chen, S. L., 110, 121, 125
 Cheo, C. C., 92, 97, 133,
 135, 141, 146, 182, 183
 Chessin, M., 178
 Chibnall, A. C., 131, 132,
 136, 139, 143
 Cholodny, N. G., 71
 Chou, T. C., 22, 23
 Chouard, P., 267, 272, 280,
 292, 297
 Chowdhury, K. A., 339
 Christian, W., 24, 27
 Christiansen, G. S., 78, 79,
 80, 89
 Christiansen-Weniger, F.,
 340
 Chroboczek, E., 292
 Chun, H. H. Q., 90
 Claes, H., 273, 274, 279,
 280, 287
 Clagett, C. O., 76, 322
 Clark, B. E., 97
 Clark, D. G., 109, 110, 116,
 120, 126
 Clark, H., 74
 Clark, J. A. B., see
 Bennett-Clark, J. A.
 Clark, L. M., 159
 Clark, T. A. B., see
 Bennet-Clark, T. A.
 Claude, A., 132, 137
 Clements, H. F., 207, 212,
 213, 215, 226
 Clements, J. R., 101
 Clendenning, K. A., 236,
 244, 245, 252, 253, 254
 Clowes, G. H. A., 81
 Cohen, S. S., 28, 46, 322,
 324, 325
 Cohn, E. J., 143
 Cohn, M., 24
 Cohn, W. E., 41
 Collander, R., 111, 112
 Colowick, S. P., 25
 Colwell, R. N., 116, 119
 Comar, C. L., 135, 136
 Commonner, B., 53, 79, 82,
 83, 179
 Compton, O. C., 207, 212,
 213, 216
 Condon, H. M., 153
 Conn, E. E., 28, 30, 237,
 307-32, 310, 312, 323
 Conner, S. D., 208
 Cooil, B. J., 119
 Cook, R. L., 207
 Cook, W. H., 155
 Coombe, B. G., 98
 Cooper, W. C., 71, 74, 97
 Copenet, M., 5
 Cori, C. F., 19, 23, 89
 Cori, G. T., 19, 21, 23, 24
 Cori, O., 324
 Correns, C. E., 290
 Cosby, E. L., 26
 Coster, C., 339
 Covo, G. A., 39
 Cowie, D. B., 202
 Cown, W. B., 153, 163
 Cox, G. A., 13
 Crafts, A. S., 94, 109, 111,
 116, 118, 119, 123, 171,
 338
 Crane, H. L., 223
 Crane, J. C., 98, 99

Crescini, F., 297
 Crook, E. M., 134, 184
 Cross, R. J., 39
 Cruess, W. V., 149, 151, 158
 Cruickshank, D. H., 146
 Cruzado, H. J., 96
 Culpepper, C. W., 160
 Cunningham, J. W., 89
 Curtis, O. F., 109, 110, 116, 118, 120, 121, 123, 126, 292

D
 Dañin, B. J., 258, 259
 Dalblom, C., 36
 Dandliker, W. B., 59
 Daughters, M. R., 156
 Davenport, H. E., 237, 251
 Davis, E. A., 95, 253
 Davis, R. E., 81
 Davison, D. C., 323
 Dawson, E. H., 149
 Dawson, R. F., 116
 Day, R., 236
 DeBusk, B. G., 319
 DeFelice, D., 161
 De Geer, E. H., 340
 Delap, A. V., 9
 del Campillo, A., see
 Campillo, A. del
 Delwiche, C. C., 133, 183
 Delwiche, E. A., 318
 Demolon, A., 11
 Denffer, D. von, 72, 97, 280, 287, 293, 294
 Dent, C. E., 326
 de Saussure, T.,
 see Saussure, T. de
 de-Sousa, A. E., see
 Esteves-de-Sousa, A.
 DeTar, J. E., 98
 de Waard, J., see Waard,
 J. de
 Dhillon, A. S., 123
 Dickens, F., 325
 Diehl, H. C., 149-70, 149,
 150, 152, 157, 158, 160,
 161, 164
 Dietrich, W. C., 158
 Dijk, P. J. S. van, 112
 Dimick, K. P., 159
 Dingle, J. H., 158
 Dische, Z., 322, 324, 325
 Dolin, M. I., 319
 Doolittle, W. T., 339
 Dostal, R., 71
 Doudoroff, M., 22, 24, 31,
 47, 48
 Douglass, A. E., 340, 341
 Douthreligne, J., 229
 Downes, W. F., 9
 Drake, M., 210
 Drosdoff, M., 213, 216, 219
 DuBois, C. W., 149
 DuBuy, H. G., 51, 137, 138,

141
 Ducet, G., 236
 Duggar, B. M., 238
 Duhamet, L., 102
 Durfee, H. K., 60, 61
 Durroux, M., 5
 Durso, D. F., 23
 Dutton, M. J., 238
 Duysens, L. N. M., 238,
 239
 Dyar, M. T., 22

E
 Eames, A. J., 95
 Eaton, F. M., 91
 Edgerton, L. J., 11, 101
 Efelkin, A. K., 290
 Egger, S. V., 60
 Eggman, L., 139, 142, 143,
 144, 145, 146
 Egle, K., 335
 Eguchi, T., 299
 Ehrmantraut, H. C., 241,
 244, 252, 253, 254
 Eidmann, F. E., 334
 Eisenmenger, W. S., 2
 Elgabaly, M. W., 12
 Ellison, J. H., 103
 Emerson, R., 238, 241, 253
 Emerson, R. L., 38, 48, 52
 Emmert, E. M., 207, 208,
 209, 214
 Eny, D. M., 309
 Epstein, E., 204
 Erdman, F. S., 149
 Eremenko, V. T., 291
 Erickson, L. C., 97, 99
 Erickson, R. O., 42, 55
 Eriksson, E., 7
 Erlandsson, S., 340
 Ernst, A., 335
 Esau, K., 109, 118, 337
 Esteves-de-Sousa, A., 73
 Evans, E. A., Jr., 310
 Evans, H., 102
 Evers, C. F., 149, 162
 Evstigneev, V. B., 257
 Eyring, H., 252

F
 Fabian, I., 273, 274, 276
 Facey, V., 100
 Fager, E. W., 250, 307,
 317, 319, 322
 Fan, C. S., 253
 Felton, M. W., 178
 Fenton, F., 157
 Feofilov, P. P., 241
 Ferri, M., 61
 Feulgen, R., 54
 Fields, M., 94
 Filippenko, I. A., 291
 Finnegan, W. J., 152, 162,
 163
 Fischer, H., 109, 121

Fisher, E., 216
 Fitzpatrick, T. B., 159
 Florschlütz, P. A., 68, 70
 Fogel, S., 82
 Fontaine, T. D., 53
 Foreman, E. M., 159
 Forrest, R. S., 28
 Förster, T., 242
 Foukkes, E. C., 309
 Frampton, V. L., 141
 Franck, J., 194, 201, 242,
 251, 252, 253
 Franklin, J., 236
 Frazier, R. R., 208
 Frear, D. E. H., 212
 Freeman, G. G., 180
 French, C. S., 238, 239,
 241, 244, 245, 246, 247,
 252, 253
 Frenkel, A. W., 236
 Frey-Wyssling, A., 91, 100,
 118, 120, 230, 232, 233,
 234
 Friedrich, J., 339
 Friess, H., 156
 Frith, H. J., 101
 Fruhstorfer, W., 308
 Fuller, H., 72
 Fullmer, F., 207
 Fulton, R. W., 179
 Fults, J. L., 90
 Funke, G. L., 281
 Furr, J. R., 97
 Fyfe, W. S., 9

G
 Gaffron, H., 242, 250, 252,
 307, 317, 319, 322
 Gall, E., 275
 Gall, H. J. F., 77
 Gallup, A. H., 62, 93
 Galos, G., 153
 Galston, A. W., 60, 67, 70,
 71, 73, 77, 90, 95, 136,
 270, 282
 Gardner, F. E., 92, 100,
 101
 Garner, W. W., 272, 274,
 275, 276
 Gassner, G., 340
 Gates, C. M., 101
 Gäumann, E., 340
 Gavrilova, V. A., 257
 Gawadi, A. G., 100
 Gehenio, P. M., 151, 152,
 153, 155, 156
 Geiger, R., 340
 Gettler, L., 230
 Geleik, H., 250
 Gentscheff, G., 274
 Gerhard, E., 287, 292
 Gerretsen, F. C., 14
 Geurten, I., 334, 336
 Ghosh, J. C., 258
 Gibbs, M., 316
 Gibbs, R. D., 337

- Gieseeking, J. E., 4
 Gilbert, B. E., 208, 214,
 217, 218
 Gilbert, G. A., 21
 Gilbert, S. G., 219
 Gilpin, G. L., 149
 Ginter, W. D., 90
 Gleen, H., 8
 Glenn, D. S., 156
 Glikman, T. S., 258
 Glock, G. E., 325
 Goddard, D. R., 17, 29, 35,
 43, 307, 310
 Goebel, C., 290
 Goldacre, P. L., 76, 95
 Goldstein, A., 66
 Gollub, M. C., 236, 310,
 311, 312
 Goodall, D. W., 207, 209,
 212
 Goodwin, R. H., 95
 Gordon, S., 143
 Gordon, S. A., 60, 61, 74,
 94
 Gorham, P. R., 245, 252
 Gorke, H., 156
 Gorodskaja, O. S., 179
 Gorter, C., 73
 Gortner, W. A., 149, 154,
 155
 Gould, W. A., 161
 Grafflin, A. L., 312, 313
 Graham, E. R., 2
 Granick, S., 132, 135, 136,
 230, 327
 Gray, G. W., 140
 Green, A. A., 23
 Green, D. E., 22, 27, 39
 Green, G. T., 27
 Green, H. A., 27
 Green, M., 72
 Greenwood, A. D., 82
 Gregory, F. G., 115, 126,
 207, 209, 212, 266, 290,
 291, 293, 294, 295, 297
 Gregory, L. E., 74, 103
 Greulach, V. A., 268, 280
 Grieve, B. J., 184
 Griggs, W. H., 98
 Gross, C. R., 149, 160
 Gründler, H., 72, 97, 280
 Guadagni, D. G., 159
 Gümmer, G., 276, 277, 282,
 299
 Günsalus, I. C., 27, 319
 Gupta, S. B. S., see
 Sen-Gupta, S. B.
 Gurevich, A. A., 246
 Gustafson, F. G., 90
 Gustafsson, A., 274
 Guthrie, J. D., 173
 Gutterman, B. M., 158
 Guyer, R. B., 161

 H
 Haas, A. R. C., 116

 Hacker, G. J., 165
 Hackett, D., 81, 83
 Haddock, J. L., 207, 216,
 224
 Hadiwidjaja, T., 184
 Hagen, C. E., 76, 204
 Hagen-Smit, A. J., 59
 Haines, R. B., 164, 165
 Halbsguth, W., 110
 Hall, A. D., 208, 212, 214,
 216, 217
 Hall, W. C., 69
 Halley, A. D., 60, 61
 Halliday, D. J., 78, 88, 95
 Hamner, C. L., 91, 94, 95,
 96, 97, 112
 Hamner, K. C., 71, 73, 212,
 268, 269, 270, 272, 275,
 276, 277, 282, 283, 286,
 287, 295, 298
 Hance, F. E., 209
 Hand, D. B., 94
 Handley, R., 200, 204
 Hanes, C. S., 17, 22, 24,
 28, 43, 44, 47
 Hanke, M. E., 327
 Hansch, C., 62, 63, 64, 67,
 68, 93, 94
 Hansen, C. M., 95
 Hanson, E. A., 132, 135,
 136, 146, 234
 Harary, I., 311
 Harder, R., 71, 72, 268,
 269, 275, 276, 277, 278,
 279, 280, 282, 283, 286,
 288, 298, 299
 Hardin, E. B., 40
 Hardin, L. J., 208, 217,
 218
 Hargiss, C. O., 165, 166
 Harley, C. P., 99
 Harmer, P. H., 4
 Harris, H. C., 122
 Harting, J., 46
 Hartley, C. W. S., 103
 Hartman, R. T., 184
 Hartmann, H. T., 98, 100,
 268
 Hartt, C. E., 110, 116
 Harvey, R. B., 156
 Haseman, J. F., 12
 Haskins, A. L., 212
 Hasse, K., 308, 326
 Hassid, W. Z., 17, 20, 21,
 22, 23, 24, 28, 31, 46,
 47, 48
 Hatcher, E. S. J., 299
 Hauschild, I., 275, 276, 282
 Haut, I. C., 161
 Havinga, E., 63
 Havis, A. L., 99
 Havis, L., 99
 Haworth, W. N., 17, 19, 47
 Haxo, F. T., 238
 Hay, R. J., 90
 Heard, C. R. C., 17, 43, 44
 Hehre, E. J., 31, 48

 Heid, J. L., 161
 Heikens, H. S., 110
 Heimbürger, G., 236, 247,
 260
 Heintze, S. G., 7, 8, 9, 11
 Heinze, P. H., 268, 270,
 282
 Heiss, R., 162
 Heitz, E., 229, 230
 Helder, R. J., 115, 127
 Helgeson, E. A., 76
 Hemphill, D. D., 98
 Hendricks, R. H., 327
 Hendricks, S. B., 273, 276,
 281, 282, 295
 Herbert, D., 311, 313
 Hesmer, H., 343
 Hester, J. B., 209
 Hestrin, S., 48
 Hewetson, F. N., 212
 Hewitt, E. J., 189, 207,
 218, 220, 313
 Hibbard, A. D., 100
 Hield, H. Z., 99, 101
 Highlands, M. E., 165
 Hildebrand, E. M., 123, 176
 Hildebrandt, A. C., 102
 Hildreth, A. C., 152
 Hill, E. V., 88
 Hill, H., 212, 213, 220
 Hill, R., 137, 235, 236, 237,
 244, 246, 251, 252
 Hirschfeld, A., 39, 43
 Hitchcock, A. E., 67, 68, 73
 Hoagland, D. R., 91, 110,
 210, 212, 216, 338
 Hobson, P. N., 19, 20, 22
 Hoehne, W., 95
 Hoffer, G. N., 208
 Hoffman, M. B., 100, 101
 Hoffman, O. L., 93
 Höfler, K., 111, 112
 Hogeboom, G. H., 132
 Hohl, L. A., 149, 154, 157,
 160
 Holden, C., 42, 55
 Holden, M., 181, 182
 Holdheide, H., 341
 Holdsworth, H., 294
 Holley, R. W., 60, 61, 94
 Holmes, F. C., 176, 181
 Holmström, A., 111
 Holt, A. S., 237, 244, 245,
 246, 247, 252, 253, 257
 Holzapfel, L., 155, 156
 Holzmann, B., 335, 336
 Horecker, B. L., 28, 322
 324, 325
 Hornberger, C. S., Jr., 319
 Horne, V., 66, 70, 94
 Hosek, M., 71
 Hougas, R. W., 175
 Hough, L., 28, 31
 Houghtaling, H. B., 299
 Howard, L. B., 156
 Howlett, F. S., 300
 Hruschka, H. W., 101

Hsiang, T.-H. T., 90, 99
Huber, B., 109, 117, 118,
119, 333-46, 333, 335,
336, 337, 338, 339, 340
Hubert, B., 232
Hudson, C. S., 321, 324
Hughes, W. L., Jr., 143
Hulme, A. C., 327
Hülsbruch, M., 111
Hurlbert, R. B., 132
Hustrulid, A., 149
Hutchins, L. M., 176
Hutner, S. H., 39, 203

I

Īarkovafa, L. M., 270
Īastreb, M. G., 290
Ichisaka, V., 208, 209
Ide, L. E., 161
Illingworth, B., 21
Inoue, Y., 22
Ionesco, H., 314
Irving, G. W., Jr., 53
Isbell, H. S., 324
Isenberg, F. M. R., 95
Iterson, W. van, 230

J

Jackson, M. L., 6
Jacobs, E. E., 243
Jacobson, L., 189-206, 200,
203, 204
Jagendorf, A. T., 90, 95,
131-48, 146
Jahnel, H., 119, 337, 338
James, G. M., 17, 26
James, G. W., 17, 24
James, H. L., 164
James, W. O., 17, 26, 29,
35, 36, 43, 44, 49, 50
Janssen, G., 210, 214
Jazewitsch, W. von, 340
Jenkins, R. R., 161
Jensen, C. O., 95
Jensen, P. B., see Boysen-
Jensen, P.
Jerchel, D., 280
Johannesen, G. A., 155
John, A., 340, 318
Johns, A. T., 318
Johnson, E. B., 88, 341
Johnson, G., 159
Johnson, I. K., 159
Johnson, J., 176
Jones, J. K. N., 28, 31
Jones, L. H., 14
Jones, L. H. P., 5, 10, 11,
12
Jones, W. W., 215
Josephson, L. M., 97
Joslyn, M. A., 149-70, 149,
150, 151, 155, 157, 158,
160, 163, 165
Junkins, S. C., 212, 213,
214

Junowicz-Kocholaty, R., 27

K

Kabat, E. A., 140
Kachan, A. A., 259
Kaila, A., 14
Kalckar, H. M., 25
Kalmus, H., 179
Kaloyereas, S. A., 153
Kandler, O., 49
Kaplan, N., 47
Karstens, W. K., 230
Kassanis, B., 146, 176,
177, 179, 180, 184
Katunskif, V. N., 281
Katz, J., 23
Katznelson, H., 178, 179
Kaufman, J., 101
Kaufman, S., 314
Kausche, G. A., 230
Ke, C. L., 254
Kearns, K., 74
Kelly, S., 81
Kennedy, E. J., 103
Kennedy, E. P., 24
Kennedy, J. S., 184
Kent, N. L., 116
Kerr, T., 91, 100
Kertesz, Z. I., 91, 100, 159
Kethley, T. W., 153, 163
Khudairi, A., 271, 279
Kielley, R. K., 25
Kielley, W. W., 25
Kiermeier, F., 158
King, H. M., 200, 204
Kisser, J., 339
Kieczkowski, A., 173, 175
Kieczkowski, J., 173
Klein, R. M., 25, 30, 309
Klein, W. H., 278
Klešnin, A. F., 281
Klevstrand, R., 321
Kliman, S., 10
Klomparsens, W., 96
Klotz, L. J., 99, 101
Knight, C. A., 174
Knorr, M. V., 254
Knott, J., 268, 272
Knowlton, G. K., 98
Knox, W. E., 27
Kobel, M., 43
Kobyakova, A. M., 25, 237
Kocholaty, R. J., see
Junowicz-Kocholaty, R.
Koenig, M. L. G., 138, 245
Koepfli, J. B., 62
Kohman, E. F., 158
Kok, A. C. A., 112
Kok, B., 249
Kolbe, R. W., 118
Konovalev, I. N., 291
Koppelman, R., 327
Korkes, S., 314, 319, 324
Kornberg, A., 25, 310, 312,
313
Kosobutskaja, L. M., 255

Kostfůchenko, I. A., 297
Kraemer, L. M., 30, 237,
310
Krahl, M. E., 81
Kramer, A., 161
Kramptitz, L. O., 319
Krantz, B. A., 207
Krantz, F. A., Jr., 161
Krasnovsky, A. A., 255,
256
Kraus, E. J., 101
Krebs, H. A., 307
Kribben, F. J., 72, 73, 300
Kristiansen, J., 236
Kriukova, N. N., 47
Krober, O. A., 116
Kröner, H., 340
Krossing, G., 237
Kruyt, W., 103
Kubota, T., 207, 212, 215
Kuchel, R. H., 146
Kuhn, R., 254
Kuiper, J., 270
Kumm, T., 245, 252
Kunkle, L. E., 149
Kunkel, L. O., 176
Kursanov, A. L., 22, 47
Kurtz, E. B., Jr., 300
Kutsky, R. J., 93, 179
Kuvaeva, E. B., 235
Kvamme, J., 76

L

Lackey, M. D., 137, 138
Ladefoged, K., 339
Lagatu, H., 208, 211, 216,
218, 223
Lalbach, F., 72, 73, 272,
300
Lampen, J. O., 322, 324,
325
Landefeldt, U. B., see
Berger-Landefeldt, U.
Lang, A., 265-308, 266,
267, 268, 270, 271, 272,
273, 274, 275, 276, 277,
278, 280, 282, 287, 289,
290, 292, 283, 294, 295,
297, 298, 299
Langner, W., 342
Lanning, M. C., 28
Lardy, H. A., 311
Larsen, H., 317
Larsen, P., 61, 94, 175
La Rue, J. L., 176
Laties, G., 137, 309
Lauffer, M. A., 173
Lawrence, N. S., 138, 245
Leaper, J. M. F., 93
Leavenworth, C. S., 90
Lebeaux, J. M., 154, 155
Leben, C., 179
Lederberg, J., 48
Lee, F. A., 154, 155, 157,
161, 165
Leeper, G. W., 1-16, 5, 8, 9,

- 10, 11, 12
 Lees, H., 6, 7
 Legault, R. R., 156
 Lehmann, H., 235
 Lehnert, I., 339
 Lehniger, A. L., 25, 51
 Leichter, H., 155, 156
 Leonhardt, H., 120, 337
 Leopold, A. C., 278, 280, 285
 Leopold, C., 71, 72
 LePage, G. A., 36
 Lerner, A. B., 159
 Leuchtenberger, C., 54
 Leviell, F., 92, 179
 Levine, M., 102
 Levitt, J., 82
 Levy, W. J., 159
 Lewis, C. C., 2
 Lewis, C. M., 238, 241
 Lewis, K. F., 320, 321
 Li, C. H., 72, 73
 Lichstein, H. C., 311
 Liebich, H., 235
 Liebig, J., 207, 217
 Lifson, N., 318
 Lilleland, O., 100, 207, 212, 213, 215, 216, 224
 Limasset, P., 92, 179
 Lincoln, R. G., 282, 283
 Linder, P. J., 123
 Lindquist, B., 341
 Lindquist, F. E., 158
 Lineweaver, H., 66
 Link, G. K. K., 25, 30, 60, 309
 Linné, C. von, 340
 Linschitz, H., 259
 Linser, H., 80, 61, 280
 Lipmann, F., 81, 322, 324, 325
 Lipshitz, R., 40
 Ljubimenko, V. N., 268
 Liverman, J. L., 69, 72, 73, 74, 268, 272, 273, 274, 278, 279, 280, 282, 283, 297, 300
 Livingston, R., 238, 241, 254, 258, 259
 Lizard, A. A., 73
 Loehwing, W. F., 268, 282
 Loesche, H. W. von, 149
 Lohmann, K., 28
 Lona, F., 72, 267, 269, 275, 276, 279, 280, 282, 283, 285, 287, 288, 298
 Long, E. M., 275, 276, 297
 Long, L., 149
 Loomis, W. D., 24, 26
 Loomis, W. F., 61
 Lorber, V., 318
 Lorenz, O. A., 119, 212, 213
 Loring, H. S., 179
 Loustalot, A. J., 96, 219
 Lovejoy, R. D., 158
 Lowe, B., 149
 Lowe, J. S., 110
 Lozano, B. A., see Arreguin-Lozano, B.
 Lucas, E. H., 94, 123
 Lucas, R. E., 212
 Luckwill, L. C., 100, 103
 Lugg, J. W. H., 131, 141
 Lumry, R., 252
 Lundegårdh, Z., 114, 115, 198, 201, 207, 212, 216, 217, 220, 224, 225
 Lusena, C. V., 155
 Lüttgens, W., 237, 245, 246, 252, 253
 Luttkus, K., 115
 Lutz, J. M., 159, 160
 Luyet, B. J., 151, 152, 153, 155, 156
 Lwoff, A., 314
 Lynch, V. H., 317
 Lynd, J. Q., 207
 Lyon, C. B., 212
 Lysenko, T. D., 297
- M
- McAlister, D. F., 116
 MacArthur, M., 154, 155
 McBrady, J. J., 259
 McCalla, A. G., 131
 McCance, R. A., 53
 McCollam, M. E., 207
 MacDougall, D. T., 336, 337, 339
 Macdowall, F. D. H., 252
 Macey, A., 19
 McGoldrick, F., 103
 McIlraith, W. J., 89
 Mack, W. B., 216, 223
 McKee, H. S., 131
 McKinney, H. H., 146, 294
 McLean, F. T., 208, 214, 217, 218
 McManus, I. R., 311
 McNair Scott, D. B., 28, 325
 McNew, G. L., 93
 McReady, R. M., 47, 48
 Macy, P., 218, 220
 Magness, J. R., 149, 160
 Magoon, C. A., 164, 165
 Mahdihassan, S., 54
 Makower, B., 159, 161
 Malan, P., 65, 66, 68, 70
 Malins, M. E., 339
 Mandeles, S., 327
 Mann, L. K., 275, 276, 300
 Mann, P. J. G., 7, 8, 9, 11
 Manning, W. M., 238
 Maramorosch, K., 171
 Markham, R., 183
 Markle, J., 116
 Marmur, J., 322
 Marsh, G. L., 151, 155, 158, 163
 Marshall, C. E., 13
 Marshall, E. R., 103
 Marth, P. C., 88, 99, 101, 103
 Martin, L. F., 146
 Maskell, E. J., 110, 116, 121, 125
 Mason, T. G., 109, 110, 116, 117, 120, 121, 125, 126
 Masterman, N. K., 149
 Masure, M. P., 158, 161
 Matruhot, L., 152
 Matthews, M. B., 323
 Matthews, R. E. F., 179, 180
 Mattson, S., 7, 10, 11
 Maume, L., 208, 211, 216, 218, 223
 Mavis, J., 161
 Maximow, N. A., 152, 157
 Mayer, J. E., 194, 201
 Mayer, M. M., 140, 159
 Mayer-Wegelin, H., 339
 Mazia, D., 82, 83
 Meeuse, B. J. D., 17, 29, 35, 51, 307, 310
 Mehler, A. H., 246, 247, 310, 313
 Melchers, G., 268, 270, 271, 272, 273, 275, 277, 278, 279, 280, 287, 289, 290, 292, 293, 295, 297
 Melin, M., 143
 Mendel, K., 334
 Meneghini, M., 133, 183
 Menke, W., 132, 135, 136, 139, 232, 235
 Menzel, R. G., 6
 Mercer, F., 179
 Mergentime, M., 157
 Metzberg, R. L., 62, 63, 64, 93, 94
 Meutémédian, A., 21, 47
 Meyer, K. H., 21
 Meyerhof, O., 27, 28, 89
 Michel, B. E., 25, 30, 79, 309
 Michel, E., 120
 Michelson, C., 24, 26
 Michener, H. D., 279
 Milad, J., 337
 Millar, F. K., 327
 Miller, G. L., 178
 Miller, I. H., 76, 90
 Millard, A., 25, 30, 51, 80, 81, 308, 319
 Millikan, C. R., 14
 Milner, H. W., 138, 245
 Milthorpe, J., 201
 Mims, V., 326
 Minarik, C. E., 95, 96
 Mirsky, A. E., 54
 Mitchell, H. L., 207, 208, 213, 214, 215, 216
 Mitchell, J. E., 94
 Mitchell, J. W., 123
 Mitchell, J. W., 78, 88, 94, 101

Molisch, H., 152, 338
 Mollard, M., 152
 Mommaerts, W. F. H., 236
 Monod, J., 48
 Moon, H. H., 99, 159, 160
 Moore, R., 116
 Morel, G., 102
 Morris, T. N., 158
 Morrison, J. F., 309
 Moškov, B. S., 268, 270,
 272, 275, 276, 281, 282,
 283, 287
 Mottern, H. H., 157
 Moulton, J., 60
 Mrak, V. G., 149
 Mühlethaler, K., 230, 233,
 234
 Muir, R. M., 62, 63, 64,
 67, 68, 93, 94
 Mulder, D., 1, 3, 6
 Mulford, D. J., 143
 Müller, R., 280
 Muller, W. H., 82
 Müller-Stoll, H., 339, 340
 Müller-Thurgau, R., 152
 Münch, E., 109, 110, 118,
 119, 120, 127, 342
 Münster, F., 208, 214
 Murneek, A. E., 59, 90,
 100, 272, 280
 Murphy, P. A., 181
 Murray, M. A., 73
 Muzik, T. J., 96
 Myers, A. T., 159, 223

N

Nadeau, A., 48
 Nance, J. F., 89
 Naylor, A. W., 95, 103,
 268, 269, 273, 282, 283
 Naylor, F. L., 300
 Naylor, N. M., 22
 Neeley, W. B., 91
 Neish, A. C., 132, 135, 136,
 235, 236, 237
 Nellis, L. F., 165
 Nelson, R. T., 97
 Nelson, W. L., 207
 Neuberg, C., 43
 Neuberger, A., 324
 Newcomb, E. H., 30, 77,
 90, 100
 Newcomer, E. H., 132
 Newman, A. S., 96
 Newton, A., 60
 Nezhgovorov, S., 237
 Nicholas, D. J. D., 207,
 209, 212, 214, 216, 218,
 220
 Nicholas, J. E., 163
 Nie, R. van, 115
 Nielsen, E. S., see
 Steemann-Nielsen, E.
 Nielsen, J. P., 161
 Nieva, F. S., see Sanchez-
 Nieva, F.

Nightingale, G. T., 207,
 212, 213, 216, 226
 Nitsch, J. P., 90, 97, 99,
 299, 300
 Nixon, H. L., 184
 Noack, K., 235
 Nord, F. F., 155, 156
 Nordal, A., 321
 Norman, A. G., 64, 69, 95,
 96
 Nossal, P. M., 312, 313,
 314, 317
 Novak, V., 2
 Nussenbaum, S., 20, 21, 47
 Nutman, P. S., 294

O

Ochoa, S., 31, 50, 247, 248,
 260, 310, 311, 312, 313,
 314, 317, 319, 325, 328
 Odland, M. L., 95
 Ogur, M., 38, 39, 42, 43,
 55
 Okunuki, K., 27, 326
 Olejnikova, T. V., 272
 Olsen, K., 77
 Olsen, R. A., 51
 Onodera, K., 22
 Oppenheimer, H. R., 334
 Oppenheimer, J. R., 241,
 245
 Ording, A., 340
 Öström, A., 322
 Ortiz, P. J., 314, 317
 Osborne, D. J., 73, 90, 97,
 98, 99
 Osipova, O. P., 235
 Osterhout, W. J. V., 196
 Osterud, C. M., 165
 Oudman, J., 111, 112, 113
 Ouellet, C., 317
 Overbeek, J. van, 66, 70,
 74, 82, 87-108, 87, 88,
 90, 91, 94, 95, 96, 97,
 102, 103
 Overstreet, R., 1, 3, 4, 10,
 12, 189-206, 200, 203,
 204
 Overton, J. B., 336
 Owen, J. C., 335
 Owen, F. V., 292, 297

P

Paddick, M. E., 10
 Paes, R., 342
 Palmquist, E. M., 121, 125
 Pardee, A. B., 231
 Pariser, R., 258
 Parker, E. R., 215
 Parker, M. W., 268, 270,
 273, 275, 276, 278, 281,
 282, 283, 295
 Patrick, A. D., 21
 Pavlinova, O., 22
 Payne, M. G., 90

Pearson, G., 210, 211
 Peat, S., 17, 19, 20, 22, 23,
 30, 31, 47
 Pedersen, K. J., 11
 Pedersen, K. O., 144
 Pederson, C. S., 161, 164
 Peech, M., 5, 220
 Pennington, M. E., 149
 Pensky, J., 17, 30, 318
 Pentzer, W. T., 101, 160
 Perrin, F., 241
 Perrin, J., 241
 Peschel, W., 338
 Pfeiffer, M., 120, 121
 Pfeiffer, T., 208, 218
 Phillips, W. R., 163
 Phillis, E., 109, 116, 120,
 121, 125, 126
 Pickels, E., 137
 Pickett, T. A., 156
 Piper, C. S., 8, 210
 Pirie, N. W., 39, 140, 143,
 145, 146, 171-88, 173,
 174, 178, 182, 183, 184
 Pisek, A., 334
 Pizer, N. H., 9
 Plagge, H. H., 149
 Plaut, G. W. E., 311
 Poignant, P., 292
 Polster, H., 334
 Pons, W. A., Jr., 53
 Ponting, J. D., 150, 158,
 159
 Popp, H. W., 95
 Porret, D., 259
 Porteous, H., 98
 Porter, H. K., 22
 Porter, K. R., 230
 Portsmouth, G. B., 9
 Potter, A. L., 48
 Potter, V. R., 132
 Pound, G. S., 176
 Powell, R. D., 245, 252
 Pratt, J. W., 321
 Prescott, S. C., 149, 164,
 165
 Preston, C., 82
 Price, C., 80, 97
 Price, C. A., 308
 Price, W. C., 137, 173, 184
 Pricer, W. E., Jr., 312
 Priestley, J. H., 339
 Prince, V. E., 99
 Proctor, B. E., 149
 Pucher, G. W., 90
 Purvis, O. N., 266, 290,
 291, 292, 293, 294, 295,
 297
 Putman, E. W., 17, 46, 48

Q

Quanjier, H. M., 181
 Quantz, L., 282
 Quastel, J. H., 65
 Quinlan-Watson, T. A. F.,
 27

R

- Raalte, M. H. van, 103
 Rabak, W., 157
 Rabideau, G. S., 110
 Rabinowitch, E. I., 137,
 229-64, 235, 240, 241,
 242, 244, 246, 250, 252,
 253, 258, 259, 260
 Racker, E., 45, 322, 324,
 325
 Ramig, R. E., 212, 218
 Rapoport, S., 36
 Raub, A., 327
 Raven, K. A., 282, 283
 Rawlins, T. E., 93, 179
 Razumov, V. J., 276
 Recknagel, R. O., 132
 Redeman, C. T., 61
 Reece, P. C., 97
 Reed, H. S., 116
 Reed, L. J., 319
 Reinders, D. E., 82
 Reiner, J. M., 190
 Reinert, J., 61
 Reman, G. H., 238
 Resende, F., 287, 300
 Reuther, W., 213, 215, 216
 Reynolds, H., 149
 Rhodes, A., 89, 103
 Rice, E. L., 97, 123
 Richtmyer, N. K., 321
 Riedel, L., 163
 Rietsema, J., 94
 Riker, A. J., 76, 90, 94
 Rippel, A., 208, 218
 Ris, H., 54
 Rischkov, V. L., 146, 179
 Roberts, E. A., 230
 Roberts, F. M., 174, 175
 Roberts, I. Z., 202
 Roberts, R. B., 202
 Roberts, R. H., 270, 272,
 287, 294
 Robertson, R. N., 51, 81,
 109, 114, 189, 199, 201,
 202
 Robinson, W. B., 161
 Rückl, B., 338
 Rodgers, P. D., 157
 Roe, J. H., 37
 Roedel, B., 126
 Roger, M., 21, 47
 Rohan, J., 94
 Rohmeder, E., 342
 Rohrbough, L. M., 123
 Romell, L. G., 337
 Roodenburg, J. W. M., 73
 Rosen, G., 42, 55
 Rosenberg, A. Y., 236
 Rosenberg, G. L., 250
 Rosenberg, J. L., 317
 Rosenberg, T., 191, 205
 Ross, A. F., 180, 185
 Rothmund, P., 254
 Rouschal, E., 109, 111, 112,
 118, 123, 124, 125, 337
 Rudney, H., 25
 Rüegger, A., 137
 Ruhland, W., 110
 Rumley, G. E., 179
 Ruska, H., 230
 Russell, E. W., 13
 Rykenboer, E. A., 165
 S
 Sachs, J., 152
 Sald, H., 110
 Sakami, W., 318
 Salles, J. B. V., see
 Veiga Salles, J. B.
 Salter, R. M., 88, 95, 98,
 101, 207, 208, 214
 Saltman, P., 17, 24, 26,
 41, 44
 Sampson, H., 92
 Samuel, G. S., 176
 Samygin, G. A., 273
 Sanchez-Nieva, F., 61, 74,
 94
 Sando, W. J., 294
 Sandström, B., 114
 Saussure, T. de, 207
 Sax, K. B., 42, 55
 Scarisbrick, R., 137, 236,
 244, 246, 251, 252
 Scarseth, G. D., 207, 208,
 210, 212, 214
 Schachman, M. K., 231
 Schales, O., 326, 328
 Schales, S. S., 326
 Schatz, A., 39
 Scheer, C. van der, 281
 Scheibe, G., 243
 Scher, R., 40, 44
 Schlenck, F., 322, 324, 325
 Schlitt, L., 280
 Schmidt, E., 119, 337
 Schmidt, G., 19
 Schmidt, W., 335
 Schmidt, W. A., 98
 Schmitz, J., 275, 276, 286
 Schmucker, T., 298
 Schnakenberg, G. H. F.,
 319
 Schneider, C., 65, 66, 68,
 70
 Schneider, H., 341
 Schneider, M. C., 319
 Schneider, W. C., 42
 Schober, R., 340
 Schou, L., 317, 322
 Schrader, F., 54
 Schratz, E., 334
 Schubert, A., 337
 Schulman, E., 340
 Schultz, E. S., 103
 Schumacher, A., 109, 337
 Schumacher, H. W., 326
 Schumacher, W., 109, 110,
 111, 117, 118, 120, 125
 Schuster, P., 28
 Schwartz, C. D., 160, 161
 Scofield, H. T., 121
 Scott, D. B. M., see
 McNair Scott, D. B.
 Scott, L. E., 161
 Scott, L. I., 339
 Scully, N. J., 276, 281
 Sechet, M., 92, 179
 Seeley, R. C., 93
 Sell, H. M., 61, 91, 94, 96,
 103
 Sen, B., 292
 Senden, H. van, 71, 72, 279,
 282, 283, 287, 288
 Sen-Gupta, S. B., 258
 Severin, H. H. P., 184
 Sexton, W. A., 103
 Shapiro, B., 114, 319
 Shapiro, S. A., see
 Avineri-Shapiro, S.
 Sharma, R. C., 103
 Shear, C. B., 223
 Shemin, D., 327
 Sherman, G. D., 4
 Shoji, K., 100
 Short, B. E., 163
 Shpolskij, E. A., 241
 Shiau, Y. G., 253
 Sickie, D., 258
 Siddiqi, M. S. H., 327
 Sideris, C. P., 90
 Sieling, D. H., 212
 Simmermacher, W., 208,
 218
 Simon, E. W., 94
 Singer, S. J., 139, 142, 143,
 144, 145, 146
 Singer, T. P., 17, 30, 318
 Singletary, C. C., 98
 Sinnott, E. W., 299
 Sipos, F., 116
 Sironval, C., 299
 Sisakyan, N. M., 25, 235,
 237
 Sitch, D. A., 23, 30
 Sjöström, L. B., 157
 Skerman, V. B. D., 8
 Skoog, F., 89, 95, 102
 Skoog, R., 65, 66, 68, 70
 Skvortcov, S. S., 276
 Slate, G. L., 161
 Sloep, A., 92, 100
 Sluisman, C. M. J., 281
 Smirnova, V. A., 179
 Smit, A. J. H., see
 Hagen-Smit, A. J.
 Smith, E. C. B., see Bate-
 Smith, E. C.
 Smith, E. L., 137
 Smith, F. F., 179
 Smith, F. G., 90, 95
 Smith, G. M., 336
 Smith, H. R., 161
 Smith, K. M., 183
 Smith, O., 103
 Smith, P., 79
 Smith, P. F., 213, 215, 216
 Smith, R. F., 245, 252

- Smith, W. H., 299
 Smyrniotis, P. Z., 28, 324
 Snyder, W. E., 73, 268,
 272, 274, 295
 Söding, H., 299
 Somers, G. F., 26, 90
 Sorber, D. G., 159
 Sousa, A. E. de, see
 Esteves-de-Sousa, A.
 Specht, A. W., 213
 Speck, J. F., 236, 310, 311,
 312, 313
 Spencer, E. L., 177
 Spencer, L. G., 103
 Spiegelman, S., 81, 190
 Spikes, J. D., 252
 Springorum, B., 280
 Stadtman, E. R., 24, 319
 Stafford, H. A., 308
 Stahler, L. M., 87, 88
 Stanier, R. Y., 231
 Stanley, W. M., 134, 178
 Stauffer, J. F., 38, 48, 52,
 253
 Steemann-Nielsen, E., 236
 Steenberg, F., 4, 5, 6, 12,
 210
 Stehsel, M., 60
 Steinbach, H. B., 116
 Steinberg, R. A., 90, 272
 Steinberger, R., 313
 Steinman, E., 230, 232,
 233, 234
 Stenlid, G., 81
 Stepka, W., 45, 49, 317
 Stern, J. R., 319
 Sterne, F., 149
 Stevens, H. B., 157
 Steward, F. C., 82, 102,
 131, 202, 326, 327
 Stewart, W. S., 97, 99, 101
 Stocking, R., 22, 154
 Stoddard, E. M., 179
 Stoll, A., 137
 Stoll, H. M., see
 Müller-Stoll, H.
 Stolwijk, J. A. J., 281
 Stout, M., 123, 282, 283,
 290, 292, 295, 297
 Stout, P. R., 1, 3, 4, 10,
 12, 82, 110, 116, 210,
 211
 Strain, H. H., 238
 Strauss, O. H., 66
 Strecker, H. J., 324
 Street, H. E., 110, 202
 Strong, L. E., 143
 Struckmeyer, B. E., 91,
 270, 272, 294
 Strugger, S., 111, 124, 335
 Strydom, J. C., 279
 Stuart, N. W., 126, 178
 Stumpf, P. K., 17-34, 17,
 22, 24, 26, 27, 28, 29,
 30, 43, 45, 49, 52, 76,
 316
 Stutz, R. E., 309, 316, 317,
 322
 Sumner, J. B., 22, 23
 Sun, C. N., 22
 Sussman, M., 81
 Sutter, E., 201
 Svedberg, T., 144
 Swaby, R. J., 5
 Swanson, C. P., 69
 Swanson, M. A., 23
 Sweeney, B. M., 51
 Swets, W. A., 100
 Swift, H., 54
 Sylvestre, G., 5
 Synerholm, M. E., 62
 Syring, R. H., 102
- T
- Taggart, J. V., 39
 Takahashi, W. N., 134, 141,
 179
 Takashima, S., 232
 Talburt, W. F., 156, 157
 Tammes, P. M. L., 112,
 121, 122, 124, 338
 Tanada, T., 238
 Tandan, K. N., 339
 Tang, Y. W., 60
 Tanko, B., 17, 24, 26, 43,
 44
 Tanner, F. W., 164
 Taravet, A., 273, 276
 Taves, C., 95
 Taylor, H. L., 143
 Taylor, J. F., 27
 Teakle, L. J. H., 3
 Teas, H. J., 60
 Teller, E., 242
 Templeman, W. G., 78, 88,
 95, 103
 Teorell, T., 191
 Terman, G. L., 212, 213,
 214
 Tetfurev, V. A., 291
 Teubner, F. G., 90
 Tewfik, S., 27, 28, 29, 30,
 43, 45, 49, 52, 316
 Thimann, K. V., 50, 51, 53,
 59, 62, 63, 67, 68, 70,
 71, 72, 77, 78, 79, 80,
 81, 83, 88, 89, 93, 94,
 95, 101, 103, 280, 308
 Thoennes, G., 153
 Thomas, J. B., 238
 Thomas, J. R., 96
 Thomas, M., 316
 Thomas, M. D., 327
 Thomas, W., 207, 216, 223
 Thomas, W. D., 179
 Thompson, B. F., 88, 341
 Thompson, H. E., 69
 Thompson, J. F., 131, 326,
 327
 Thornthwaite, C. W., 335,
 336
 Thornton, S. F., 208
 Thung, J. H., 230
- Thung, T. H., 184
 Thurgau, R. M., see
 Müller-Thurgau, R.
 Thurlow, J., 71, 72, 73, 74,
 270, 279
 Timiriazov, K. A., 254
 Timm, E., 235
 Timofeeva, I. V., 235
 Tingley, M. A., 119
 Tinsley, T. W., 178
 Tio, M. A., 14
 Tiselius, A., 23
 Todd, G. W., 90
 Tolbert, N. E., 175, 317,
 322
 Tolmach, L. J., 31, 50,
 247, 260, 314
 Torda, C., 311
 Torriani, A. M., 48
 Tracey, M. V., 181, 182
 Tranquillini, F., 334
 Tremblay, F. T., 212, 217,
 220, 224
 Tressler, D. K., 149, 157,
 162
 Treumann, B., 76
 Trocmé, S., 5, 13
 Tsui, C., 102
 Tukey, H. B., 95, 299
 Tumanov, I. I., 73
 Turk, L. M., 207
 Turner, J. S., 44
 Turner, W. P., 157
 Tyner, E. H., 217, 218,
 220, 224
- U
- Uchiyama, A., 258
 Ulrich, A., 207-28, 207,
 209, 210, 212, 213, 214,
 215, 216, 217, 218, 219,
 220, 222, 224
 Umbach, G., 155
 Umbreit, W. W., 36, 37,
 38, 39, 44, 48, 52, 53,
 253
 Utter, M. F., 311, 318,
 320, 323, 328
- V
- Vageler, P., 2
 Vahtras, K., 7
 van Andel, O. M., see
 Andel, O. M. van
 Vandecaveye, S. C., 212,
 218
 Van der Marel, H. W., 2
 van der Scheer, C., see
 Scheer, C. van der
 van der Veen, R., see
 Veen, R. van der
 Van der Want, J. P. H.,
 179
 van Dijk, P. J. S., see
 Dijk, P. J. S. van

- Van Eseltine, W. P., 165
 van Iterson, W., see
 Iterson, W. van
 van Nie, R., see
 Nie, R. van
 van Overbeek, J., see
 Overbeek, J. van
 van Raalte, M. H., see
 Raalte, M. H. van
 Vanselow, K., 338
 van Senden, H., see
 Senden, H. van
 Vatter, A., 230, 233
 Vaughn, J. R., 96
 Vavilov, S. I., 241
 Vecher, A. S., 235
 Veen, R. van der, 249
 Veiga Salles, J. B., 310,
 311, 314, 317, 325
 Veldstra, H., 63, 94,
 103
 Velez, I., 74
 Vendrely, C., 54
 Vendrely, R., 54
 Vennesland, B., 28, 30,
 236, 237, 307-32, 310,
 312, 319, 323, 328
 Verkeerk, K., 297
 Vermeulen, D., 238
 Vickery, H. B., 90, 131
 Vishniac, W., 31, 50, 52,
 247, 248, 260, 314, 317
 Volz, S., 5
 Vojnovskaja, K. K., 256
 von Denffer, D., see
 Denffer, D. von
 von Jazewitsch, W., see
 Jazewitsch, W. von
 von Linné, C., see
 Linné, C. von
 von Loesecke, H. W., see
 Loesecke, H. W. von
 von Wettstein, W., see
 Wettstein, W. von
 von Witsch, H., see
 Witsch, H. von
 Voss, J., 294
 Vyvyan, M. C., 99

 W
 Waard, J. de, 68, 70, 73
 Wadsley, A. D., 9
 Wagenknecht, A., 60, 76,
 90, 157
 Wain, R. L., 73, 93, 97,
 98, 99
 Walker, J. C., 176
 Walkley, A., 9
 Walkley, J., 126
 Wall, M. E., 220
 Wallace, G. I., 164
 Wallihan, E. F., 218
 Wallrabe, E., 281, 282
 Walter, H., 340
 Warburg, O., 237, 245, 246,
 250, 252, 253
 Ward, A. C., 161
 Wardlaw, C. W., 102
 Warburg, O., 24, 27
 Warner, K. F., 149
 Warren, G. F., 98
 Wasicky, R., 95
 Wasscher, J., 101
 Wassink, E. C., 238, 281
 Watson, D. J., 181
 Watson, M. A., 181, 184
 Watson, T. A. F. Q.,
 see Quinlan-Watson,
 T. A. F.
 Watt, D., 319
 Waygood, E. R., 236
 Wayrynen, R. E., 252
 Weaver, R. J., 98
 Weber, R. P., 60
 Webster, J. E., 36
 Weck, H., 338
 Weeks, D. C., 114, 201,
 202
 Wegelin, H. M., see
 Mayer-Wegelin, H.
 Wegener, J. B., 157
 Weibull, C., 23
 Weier, E., 230
 Weier, T. E., 154
 Weill, B. H., 149
 Weindling, R., 178, 179
 Weinhouse, S., 320, 321
 Weinland, H., 299
 Weintraub, R. L., 64, 94,
 95, 96, 123
 Weiser, R. S., 165, 166
 Weiss, J., 258
 Wellenhofer, W., 340
 Wellensiek, S. J., 297
 Weller, L. E., 103
 Weller, S., 252
 Welsh, F. M., 174
 Weniger, F. C., see
 Christiansen-Weniger, F.
 Went, F. W., 60, 62, 88,
 94, 101, 103, 114, 117,
 281, 300
 Werle, E., 326, 327
 Westall, R. G., 159, 327
 Wester, H. V., 101
 Westheimer, F. H., 313
 Westphal, M., 282, 288
 Wetmore, R. H., 102
 Wettstein, W. von, 342
 Whalley, B. E., 341
 Whatley, F. R., 44, 50,
 245, 251, 252, 312,
 315
 Whelan, W. J., 19, 20, 21,
 22, 23
 Whistler, R. L., 23
 Whitcombe, J., 154, 155
 White, D. G., 97
 Whitehead, M. R., 101
 Whiting, A. G., 73
 Whitt, C. D., 12
 Widdowson, E. M., 53
 Wiedemann, E., 137
 Wiegand, E. H., 149, 157,
 160
 Wiersum, L. K., 115, 122,
 270
 Wiklander, L., 4
 Wilbur, J. S., 159
 Wildman, S. G., 25, 39, 42,
 43, 44, 59, 60, 61, 79,
 80, 92, 97, 131-47, 133,
 134, 135, 139, 141, 142,
 143, 144, 145, 146, 147,
 182, 183, 237, 281
 Wilkins, M. J., 199, 202
 Wilkinson, I. A., 19
 Wilks, J. M., 174
 Williams, K. T., 321
 Williams, R. F., 126
 Williams, W. O., 98
 Williamson, C. E., 185
 Wilske, C., 70
 Winter, A. R., 149
 Winter, J. D., 149
 Winterstein, A., 254
 Withner, C., 44
 Withrow, A. P., 268, 269,
 275, 281, 282, 287
 Withrow, R. B., 268, 269,
 273, 275, 276, 281, 282,
 287
 Witsch, H. von, 268, 269,
 278, 279, 282, 298
 Wittenberg, J., 327
 Wittwer, S. H., 59, 61, 88,
 95, 97, 98, 101, 103,
 299
 Wohl, K., 242
 Wolf, B., 208, 209
 Wolf, J., 318
 Wolff, E., 214
 Wolff, H. G., 311
 Wood, H. G., 311, 318, 320,
 323, 328
 Wood, J. G., 131, 136,
 146
 Woodroof, J. G., 149, 153,
 154, 162, 164
 Woods, M. W., 137, 138,
 141, 179
 Woolrich, W. R., 163
 Working, E. B., 336
 Wort, D. J., 294
 Wyatt, G. S., 54
 Wyckoff, R. W. G., 134,
 137, 230, 233
 Wynd, F. L., 181

AUTHOR INDEX

357

Wyssling, A. F., see
Frey-Wyssling, A.

Y

Yarwood, C. E., 180

Yin, H. A., 22

Youden, W. J., 173

Young, H. Y., 90

Young, J. O., 299

Young, L., 63

Young, R. A., 11

Young, V. K., 238, 239,
241

Z

Zanoni, G., 299

Zaruballo, T. F., 297

Zdanova, L. P., 279

Zimmerman, P. W., 62, 63,
67, 68, 73, 94, 99

SUBJECT INDEX

A

- Abscission
 - auxin and, 92
 - of fruit, 99-102
 - see also Fruit drop
- Accelerated diffusion, translocation and, 120
- Accumulation
 - of ions, 189-206
 - see also Ion absorption
- Acetaldehyde, formation of, from pyruvate, 318-19
- Acetate, micronutrient supply and, 11
- Acid soils, manganese and, 9
- Action spectra, of photoperiodism, 281-82
- Activity measurements
 - nutrient availability and, 13-14
 - see also Micronutrients
- Adenosinediphosphate
 - estimation of, 35-42
 - see also Phosphorylated compounds
- Adenosinetriphosphate
 - auxins and, 81
 - estimation of, 35-42
 - photosynthesis and, 52
 - sucrose synthesis and, 48
 - see also Phosphorylated compounds
- Adenylic acid, estimation of, 35-42
- Adenykinase, 26
- Adesine, nutrient supply by, 2
- Aging
 - nutrient availability and, 11-13
 - definition of, 12
 - soil surface area and, 12, 13
 - see also Micronutrients
- Aldolase, 27-28
 - distribution of, 27
 - pentose synthesis and, 28
 - properties of, 27-28
 - zinc and, 27
- Alkali soils, manganese and, 9
- Amino acid decarboxylases, 326-27
 - see also Glutamic acid decarboxylase
- Amino acids
 - ion accumulation and, 202-3
 - malic enzyme and, 312
- Amylase, auxin and, 77
- Amylopectin, synthesis of, 19-22
- Amylose, synthesis of, 19
- Anachris, bleeding in, 122-23
- Analysis, of plants, 207-26
 - see also Plant analysis
- Anion exchange resin, phosphorylated compounds and, 41-42
- Anion respiration, anion transport and, 114
- Anions, absorption of, 198-99
- Antiauxins
 - action of, 95
 - agricultural use and, 69-70
 - auxin research and, 70
 - from auxins, 69
 - cinnamic acid, 66-67
 - criteria of, 65
 - dichloranisol, 67
 - flowering by
 - pineapple and, 74-75
 - 2,3,5-triodobenzoic acid, 73
 - in Xanthium, 72-73
 - γ -phenylbutyric acid as, 65-66
 - roots and, 66-69
 - 2,3,5-triodobenzoic acid, 67-68
- Apple, preharvest drop in, control of, 101
- Arsenate
 - inhibition by, of growth and respiration, 80
 - replacement of phosphate by, 23-24
- Arsenite, inhibition by
 - of growth, 51
 - of growth enzyme, 53-54
 - of growth and respiration, 80
 - of phosphorous turnover, 57
- Arsenolysis, 23-24
- Ascorbic acid oxidase, auxin and, 77, 90
- Ash, in chloroplasts, 235
- Asparagine, translocation of, 111-12
- Assimilate flow
 - in trees, 336-38
 - see also Trees
- Auxins
 - action of, 89
 - in abscission, 92
 - antiauxins and, 95
 - in apple thinning, 91-92
 - on enzyme systems, 75-77
 - inhibition of, 94-95
 - in June drop, 91
 - molecular structure and, 93-94
 - pH and, 94
 - on plant composition, 78-79
 - on respiration and growth, 79-82
 - viruses and, 92-93
 - on water uptake, 82-83
 - agricultural uses of
 - in brush control, 88
 - extent of, 87-88
 - in fruit drop control, 97-102
 - in fruit formation, 88
 - in organ formation and development, 102-3
 - physiological basis and, 87-103
 - in weed control, 87-88, 95-97
 - alteration to antiauxins, 69
 - amylose activity and, 77
 - ascorbic acid oxidase and, 77, 90
 - auxin antagonists and, 65-71
 - calcium-sparing and, 91
 - catalase and, 77, 90-91
 - enzyme activity and
 - table of, 76
 - tissue treatment and, 77
 - enzymes and, 90-91
 - flower formation and
 - delay of, 97
 - inhibition of, 97
 - in pineapple, 97
 - in sugar beet, 97
 - flowering and, 299-300
 - indeterminate plants and, 72
 - inhibition of, 71-73
 - long-day plants and, 72
 - promotion of, 73-74
 - short-day plants and, 71-72
 - flower types and, 97-98
 - fruit crop control by, 97-102
 - fruit drop and, 99-102
 - fruit growth control by, 99
 - fruit set and, 98-99
 - basis of, 99
 - in figs, 98
 - in pomaceous fruits, 98

in various crops
fruit thinning and, 99
growth and development
and, 102-3
mitochondria and, 80
protein synthesis and,
78-79
rooting and, 102-3
sprout inhibition and,
103
in vitro tissues and, 102
native
identification of, 59-60
nature of, 59-62
nonindoleacetic acid, 61
organic acid metabolism
and, 89-90
pectins and, 91-92
phosphate metabolism and,
89
phosphorylase activity
and, 77
phosphylating systems
and, 81
photoperiodic induction
and, 279-80
pineapple and, flowering
of, 74
preharvest drop and, 100-2
reactivity of, 63-65
roots and, 68-69
structure of, activity and,
62-65
virus infection and, 184-85
water uptake and, 82-83
2,4-dinitrophenol and,
83
respiration and, 82
weed control and, 95-97
see also Antiauxins; Fruit
drop, prevention of;
Pectins; Photoperiodic
induction; Potato
sprouting; and Weed
control
Azide, inhibition of respira-
tion by, 201

B

Bacteria, photosynthesis in,
317-18
Bacteriochlorophyll, 231,
238
Balanced nutrient concentra-
tion, concept of, 222-
24
Barium, phosphorylated
compounds and, 36-37
Bark
respiration of, 336
transpiration of, 336
tree rings in, 340
water content of, 337
Bast glomeruli, translocat-
ion and, 117
Biotin, β -decarboxylations

and, 311
Bleeding
in Arachis, 122-23
in palms, 121-22
Blue-green algae
chlorophyll in, 231
phycocyanin in, 231
pigment particles in, 231
Bound water, colloids and,
156
Breeding, of trees, 341-43
see also Trees, breeding
of
Browning, in fruit products
melanin and, 158-59
phenolic compounds and,
159
polyphenoloxidase and,
158-59
tannins and, 159
Brush, control of, 88
Bytownite, nutrient supply
and, 2

C

C₂ units
acetaldehyde and, 318-19
dicarboxylic acid cycle
and, 320-21
glycolic acid metabolism
and, 322-23
phosphoglyceric acid and,
and, 319-20
photosynthesis and, 318-
20
from pyruvate, 318-19
ribulose and, 321-22
sedaheptulose and, 321-22
C₃ sugars
transformations of, 26-31
see also Glycolysis
Caffeine, translocation of,
111-12
Calcium
exchangeable ions and, 3
not in sieve tubes, 116-17
source of, in soils, 2
Cambium, tree growth and,
339, 341
Carbohydrates, virus infec-
tion and, 181-82
Carbon assimilation, 229-61
see also Photosynthesis
Carbon monoxide, inhibition
of respiration and ion
accumulation by,
201-2
Carboxylases, 30
in plants, 307-28
 β -carboxylases
 β -keto acids and, 313-14
light absorption and,
313-14
metal enolates and,
313-14
metal ion catalysis and,
313-14

β -carboxylations, functions
of
in photosynthesis, 317
in succulents, 316
in various plants, 316
Carotenoids
photosynthesis and, 238
in purple bacteria, 231
Carriers, ion absorption
and, 200, 203-4
Catalase
auxin and, 77, 90-91
off-flavors and, 158
Cations
absorption of, 199
malic enzyme and, 314
Cell walls, protein extrac-
tion and, 132
Chelate bonds, ion absorp-
tion and, 203
Chelation
ferric citrate and, 11
ferric tartrate and, 11
nutrient supply in soils
and, 5
Chlorella
Hill reaction in, 253-54
phosphorylated compounds
in, 38
Chloride, translocation of,
112
Chlorobenzoic acids, auxin
activity of, 64, 94
Chlorophyll
in blue-green algae, 231
colloidal, 243-44
function of, in photosyn-
thesis, 259-60
in grana, 234-35
primary photosynthetic
pigment
action as, 240-41
chlorophyll-d as, 238-
40
evidence for, 238
phycobilins and, 241
red shift of, 243
Chlorophyll-d, photosynthe-
sis and, 238-40
Chlorophyll solutions
oxidation of, 258-60
photochemistry of, 254-60
reduction of
by ascorbic acid and
light, 255-56
diphosphopyridinenucle-
otide and, 256
methyl red and, 257-58
reversal of, 256-57
solvents and, 256
by zinc and acetic acid,
254-55
Chloroplastin, 232
Chloroplasts, 229-61
amino acids in, 235
ash in, 235
chloroplastin in, 232

- composition of, 136-37,
235-37
nitrogen deficiency and,
235
electron micrographs of,
233-34
enzymes in, 235-37
carbonic anhydrase and,
235-36
carboxylases, 236
catalase, 237
cytochromes, 236-37
diphosphopyridinenucleo-
tide and, 237
hydrogenylase, 236
malic enzyme, 236
polyphenol oxidase, 237
proteases, 237
triphosphopyridinenucle-
otide and, 237
grana in
blue-green algae, 231
chlorophyll molecules
in, 234-35
number of, 230
proteoidic and lipidic
layers in, 232-33
purple bacteria, 231
size of, 230
ultracentrifugation of,
231
green algae in, 230
electron micrographs
of, 230
location of, 230
Hill reaction and, 244-54
isolated, reactions of,
244-54
isolation of
contamination in, 136
difficulties in, 135-36
intact, 135
surface active agents
and, 137-38
lamellae of, 233-34
lipoids in, 232-33, 235
membrane of, 233
pigments in, functions of,
237-44
proteins in, 137, 232-33,
235
quantity in leaf cells, 136
stroma of, 229
see also Hill reaction; and
Photosynthesis, pig-
ments and
Chlorosis, iron and, 10
Chromatographic analysis,
phosphorylated com-
pounds and, 40-41
Chromatophores, in bacteria,
231
Cinnamic acid, antiauxins
and, 66-67
Circulation systems, trans-
location and, 127
Citric acid cycle, see
Krebs cycle
Citrus, preharvest drop in,
control of, 101
Cladotopsis, in trees, 340
Cobalt, source of, in sand,
2-3
Coccarboxylase, in glycoly-
sis, 44
Coenzymes, phosphorylated,
44
Collection time, for plant
analysis, 213
Colloids, freezing of
aggregation and, 155-56
bound water and, 156
free water and, 156
pH and, 156
plant sap and, 157
precipitation and, 156
sugar solutions and,
157
Competition, between ions,
203-4
Complex formation
in soils
chelation and, 5
covalent links in, 5
organic matter and, 6
pyrophosphate and,
6-7
X-hypothesis and, 6
nutrient availability and,
3-5
see also Micronutrients
Composition of plant
auxins and, 78-79
see also Auxins, plant
composition and
Copper
distribution of, in plant,
210
exchangeable ions and,
4
phosphofructokinase and,
26
source of, in sand, 2-3
triosephosphate dehydro-
genase and, 28
Covalent links, nutrient sup-
ply in soils and, 5
Critical nutrient concentra-
tion
concept of, 217-18
definition of, 218
determination of, 219-20
factors affecting, 216-22
nutrient balance and, 222-
24
Cyanide
growth inhibition by, 80
respiration and, 201
sucrose synthesis and, 48
Cytochrome, ion absorption
and, 198-99
Cytochrome oxidase, anion
transport and, 114
Cytochrome system, phos-
phorous esterification
and, 50-52
Cytoplasmic proteins
color of, 141
composition of
in dicotyledenous leaves,
141-42
by electrophoresis, 141-
42
Fraction I in, 142
Fraction II in, 142
in monocotyledenous
leaves, 142-43
by ultracentrifuge, 142
definition of, 139
Fraction I in
compounds in, 145-46
isolation of, 143-45
properties of, 145-46
function of, 148
preparation of
problems in, 139
scheme for, 139-41
variations in, 146
viruses and, 146-47
- D
- Daylength
flowering and, 266-90
see also Photoperiodism
 β -decarboxylations, biotin
and, 311
Deficiencies, of nutrients
correction of, 224
plant analysis and, 220
Desoxyribosenucleic acid
estimation of, 42-43
nuclei and, 55
Dextrins
in amylopectin synthesis, 20
as primers, 23
Dicarboxylic acid cycle
evidence for, 320
photosynthesis and, 321
Dichloranisol, antiauxins
and, 67
2, 4-Dichlorophenoxyacetate
brush control and, 88
translocation of, 123
weed control, 87-88, 95-97
see also Weed control
Diffusion pump, osmotic,
194-96
2, 4-Dinitrophenol
growth and respiration and,
81
growth substance action
and, 51
ion accumulation and, 51,
202
phosphorous esterification
and, 51
protoplasmic streaming
and, 51
Diphosphopyridine nucleotide
chlorophyll and, 255-56

- estimation of, 35-41
in glycolysis, 44
photosynthesis and, 50, 52
Disaccharides
 synthesis of, 46-48
 see also specific compounds
Disease
 virus, physiology of, 171-85
 see also Virus
Drosera, translocation in, 112-13
Drought, tree rings and, 340-41
Dry plant material, analysis of, preparation for, 209
Dying of larches, 342
- E
- Elements, cycle of, in soil, 1
Energetics, of ion absorption, 204-5
Energy
 absorption of, for photosynthesis and, 241-44
 resonance migration of, photosynthesis and, 241-44
Enolase, inhibition by fluoride, 30
Enzyme action, ion absorption and, 204
Enzymes
 auxin and, 75-77
 carboxylating
 photosynthesis and, 307
 in plants, 307-28
 in chloroplasts, 235-37
 in glycolysis, 17-31
 off-flavors and, 158
 plants, yeast, animals compared, 17-19
 virus infections and, 180-81
 see also Auxins, enzyme activity and; Chloroplasts, enzymes in; Glycolysis; and specific enzymes
Epidermis, of root, ion absorption and, 114-15
Exchange, of ions, 190
Exchangeable ions
 adsorbents and, 5
 aluminum and, 5
 calcium carbonate and, 5
 determination of, 3-5
 extraction of, 3-5
 micronutrients and, 3-5
 nutrient availability and, 3-5
 see also Micronutrients
Excitation energy, photo-
 synthesis and, 241-44
Exudate, from trees, composition of, 338
Exudation, from sieve tubes, 118-19
- F
- Flavor, in frozen products, 158
Floral initiation
 auxins and, 71-75, 279-80
 definitions and, 266-67
 flowering and, 298-99
 relation of, 265-66
 of long-day plants, 271-75
 nature of, 289
 of short-day plants, 275-77
 self-perpetuating effects in, 296-98
 see also Long-day plants; and Self-perpetuating effects
Floral stimulus
 evidence for, 287-90
 florigen and, 287
 flower hormone and, 287
 formation of
 metabolism and, 278
 photosynthesis and, 278
 stages in, 277-78
 movement of, 282-84
 inhibition of, 282-83
 nature of, 282
 path of, 282
 rate of, 283-84
 nature and action of, 287-90
 vernalin, 293-94
 in vernalization, 292-94
Florigen, 287
Flower formation
 auxins and, 97-98
 see also Auxins, flower formation and
Flower hormone, 287
Flowering
 antiauxins and, 73-74
 auxins and, 71-75
 later stages of, 298-300
 auxins and, 299-300
 floral initiation and, 298-99
 nature of, 298
 sex differentiation in, 300
 physiology of, 265-300
 stages of, 265
 relation of, 265-66
 see also Auxins, flowering and; Floral initiation; Photoperiodism; and Vernalization
Flower sex, auxins and, 97-98
Fluorescein, transport of, 124-25
Fluorescence, in photosynthesis, 238-44, 253-54
Fluoride
 enolase and, 30
 pyruvate formation and, 43
Foliar diagnosis, 223
Formative effect, auxins and, 90
Fraction I proteins, from leaves, 143-46
Free water, colloids and, 156
Freezing
 of colloids, 155-57
 of plant products, methods of, 162-63
 of plant tissues
 coagulation and, 153
 ice formation in, 151-52
 microscopic studies of, 154
 pressures during, 152-53
 results of, 151-55
Fresh plant material, analysis of
 difficulties in, 208-9
 variability in, 208-9
Frozen plant products
 quality of, 161
 varieties for, 159-60
Fructose diphosphate
 estimation of, 35-41
 glycolytic breakdown of, 29
 nonglycolytic breakdown of, 29
Fructose-6-phosphate, estimation of, 35-41
Fruit drop
 prevention of, 100-2
 action of auxins in, 100-1
 in apples, 101
 in citrus fruit, 101
 in holly, 102
 in pears, 101
 promotion of, 100
 types of, 99-100
Fruits
 analysis of, 212-13
 browning of, 158-59
 growth of, auxins and, 99
 thinning of, 100
Fruit set
 auxins and, 98-99
 see also Auxins, fruit set and
 Fucosanthol, photosynthesis and, 238
- G
- Gas exchange, of trees,

- 333-36
- Genetics
of trees, 341-43
see also Trees, breeding of
- Geochemical approach, nutrient availability and, 3
- Gluconic acid, from glucose-6-phosphate, mechanism of, 324
- Glucose-1-phosphate, estimation of, 35-41
- Glucose-6-phosphate
estimation of, 35-41
mechanism of, 324
oxidative degradation of, 323-26
pentoses from, 324
- Glutamic acid decarboxylase
distribution of, 326
function of, 327
product of, 326-27
- Glycolic acid
oxidation of
enzyme in, 322
formate from, 322-23
steps in, 322
photosynthesis and, 322-23
- Glycolysis
alternate pathway for, 29, 45
- C₃ sugar transformation
alcohol dehydrogenase and, 30
aldolase and, 27-28
carboxylase and, 30
enolase and, 30
isomerase and, 28
lactic dehydrogenase and, 30
malic enzyme and, 30
mitochondrial system and, 30
phosphopyruvic transphosphorylase and, 30
transaminase systems and, 30
triosephosphate dehydrogenase and, 28
- hexose phosphate interconversion in, 24-26
hexokinase and, 25
myokinase and, 25-26
phosphoglucumutase and, 24-25
phosphohexoisomerase and, 26
phosphorylated coenzymes in, 44
phosphorylated compounds in, 43-46
photosynthesis and, 30-31
reversal of, in photosynthesis, 49-50
- scheme of, 17-19
starch formation in, 19-24
phosphorylase and, 22-24
Q-enzyme and, 19-22
table of, 44
triosephosphate dehydrogenase and, 45-46
- Grafting, of forest trees, 342
- Grana
in chloroplasts, 229-35
see also Chloroplasts, grana in
- Growth
auxins and, 79-82
of fruits, auxins and, 99
inhibition of, 51, 80, 95
nutrients and, 217-26
phosphorylated compounds and, 52-54
protein synthesis and, auxins and, 78-79
trees and, 338-41
see also Auxins, growth and; Critical nutrient concentration; Phosphorylated compounds, growth and; and Trees
- Growth enzyme, 53-54
- Growth substance action, inhibition of, 51
- Growth substances
agricultural uses, 87-103
nature of, 59-62
see also Auxins
- Guaiacol, in ion transport models, 196-98
- H
- Heat therapy
virus diseases and, 176-77
see also Virus infection
- Heat treatment, off-flavors and, 158
- Heterosis, in trees, 343
- Hexokinase, 25
- Hexose phosphates
interconversion of, 24-26
see also Glycolysis
- Hill reaction
in *Chlorella*, 253-54
quantum requirement of, 253
quinone and, 253-54
chloroplasts for
chloride and, 245-46
preparation of, 245
preservation of, 245-46
sources of, 245
inhibition of, 252
isotopic oxygen in, 244
kinetics of, 244, 251-53
malic acid formation and, 247-48
maximum rate of, 252
- measurements of, 251-52
methemoglobin and, 251
oxidants for, 251
cytochromes as, 246-47
nonbiological, 246
potentials of, 246-47
pyridine nucleotides as, 246-51
quantum yield of, 252
- Holly, fruit and leaf drop of, control of, 102
- Hydrogen acceptors, photosynthesis and, 248-49
- Hydrogen-ion concentration
auxin activity and, 94
colloids and, 156
- Hypersthenes, nutrient supply and, 2
- I
- Ice formation, in plants
control of, 155
death from, 152
effects of, 151-52
pressure from, 152-53
- Indeterminate plants
flowering, auxins and, 72
see also Photoperiodism
- Indoleacetic acid
in cabbage, 61
identification of, 59-62
in oats, 61
synthesis of, 60
see also Auxins
- Indoleacetic acid oxidase, 94
- Inhibition
of Hill reaction, 252-53
of ion accumulation, 201-2
of photosynthesis, 252-53
of potato sprouting, 103
of respiration, 201-2
of root growth, 102
of translocation, 113-14
of virus infection, 179-80
- Inhibitors
competitive, definition of, 65
of enolase, 30
of growth, 80, 81, 95
of growth enzyme, 53-54
of growth substance action, 51
of hexokinase, 25
of inorganic phosphorus esterification, 37
of ion accumulation of phosphofructokinase, 26
of phosphorylase, 22
of photosynthesis, 49
of protoplasmic streaming, 51
of pyruvate formate, 43
of radiophosphorus turnover, 51
of sucrose synthesis, 48
of triosephosphate

dehydrogenase, 28-29, 45-46
 Inorganic orthophosphate, estimation of, 35-41
 Inorganic phosphorous esterification, inhibition of, 51
 Iodoacetamide, triosephosphate dehydrogenase and, 28, 45-46
 Iodoacetate
 growth and, 80
 growth enzyme and, 53-54
 photosynthesis and, 48
 sucrose synthesis and, 48
 triosephosphate dehydrogenase and, 45-46
 Ion absorption
 anions and, 198-99
 carriers in
 enzyme action and, 204
 ion competition and, 203-4
 cations and, 199
 cytochrome system and, 198-99
 energetics of, 193, 196
 energy relations in, 204-5
 exchange and, 190
 facts about, 189-90
 ion influence on, 190
 iron and, 198-99
 kinetic analysis of, 190-91
 Lundegårdh's mechanism, 198-200
 mechanisms of, types of, 189
 metabolic activity, 189-90
 models of, 191-200
 by Franck and Mayer, 194-96
 by Osterhout, 196-98
 partial equilibrium, 191-93
 by Rosenberg, 191-94
 stationary equilibrium, 193-200
 nature of, 190-91
 oxygen and, 199
 potassium and, 190-91
 respiration and, 198-99
 by roots, 200-3
 amino acids and, 202-3
 anions and cations and, 201
 carrier substances and, 200
 chelate bonds and, 203
 cytochrome and, 201-2
 formulation of, 200-1
 inhibition of, 201-2
 ion carriers in, 203
 Lundegårdh hypothesis and, 201-2
 mechanisms of, 189-206
 metabolism and, 203
 oxygen and, 200
 permeability and, 200

secretion and, 203
 selectivity of, 190
 sodium and, 190-91
 Ion accumulation, inhibition of, 51
 Ion transport
 in roots, 114-16
 see also Translocation, in roots
 Ions, in catalysis of β -carboxylases, 313-14
 Ions, exchangeable, micro-nutrients and, 3-5
 Iron
 absorption of, 10
 availability of, oxidation state and, 8, 10
 chlorosis and, 10
 ion accumulation and, 198-99
 supply of
 ferric citrate and, 11
 ferric tartrate and, 11
 ferrisilicate and, 11
 Isocitric dehydrogenase
 diphosphopyridine nucleotide and triphosphopyridine nucleotide specificity of, 312-13
 distribution of, 312
 reaction of, 312
 Isomerase, 28
 Isophosphorylase, 21
 starch synthesis and, 47

J

Juices, leakage of, during freezing, 153-54

K

β -keto acids, decarboxylation of, 313-14
 Killing, by cold, 151-55
 Krebs cycle
 in *Chlorella*, 309
 cytochrome system and, 51-52
 metabolism and, 308-9
 mitochondria and, 308
 in mung bean, 308
 phosphorous esterification and, 51
 in plants, evidence for, 307-9
 succinic dehydrogenase and, 308
 in yeast extracts, 309

L

Labile phosphorous, significance of, 36
 Larches, dying of, 342
 Latex flow, mechanism of, 118

Leaf-hoppers, virus infection of host plant and, 184
 Leaf proteins
 in chloroplasts, 135-38
 in cytoplasm, 139-47
 extraction of
 cell rupturing and, 134
 cell walls and, 132
 particulate structures and, 132
 problems in, 132-34
 vacuoles and, 133-34
 in mitochondria, 138
 in particles, 134-39
 definition of, 134-35
 nature of, 134-35
 virus infection and, 182-83
 see also Chloroplasts; and Cytoplasmic proteins

Leaves

aldolase in, 27
 long-day plants and, 272-73
 nutrient balance in, 223
 photoinduction in, 269
 photoperiodism and, 285
 transport from, 125-26
 Light, virus infection and, 174-75
 Lipoids, in chloroplasts, 232-33, 235
 Lithium, translocation of, 111
 Long-day plants
 floral initiation in, 271-75
 leaves and, 272-73
 light and, 273-75
 light periods for, 272
 flowering of, auxins and, 72
 Low temperature preservation
 basis of, 150
 colloids and, 155-57
 color and flavor changes during, 157-59
 enzymes and, 157-58
 ice formation in, 141-45
 liquid environment and, 151
 maturity and, 161-62
 methods in
 factors in, 162-63
 types of, 162
 microorganisms and, 164-66
 storage death of, 165-66
 temperature and, 165
 nonbrowning peach varieties and, 161
 pectinic acids and, 157
 storage and distribution in, 163-64
 sugar and, 157
 temperatures for, 150
 undesirable effects in, 150-51
 varietal adaptation to, 159-60
 see also Browning, of

- fruits; Freezing; Ice formation; and Off-flavors
 Lundegårdh hypothesis of ion absorption, 198-200, 201-2
- M**
- Magnesium**
 exchangeable ions and, 4
 source of, in soils, 2
- Malic enzyme**
 amino acids and, 312
 biotin and, 311
 distribution of, 310
 manganese and, 312
 monovalent cations and, 314
 oxaloacetic carboxylase and, 310
 photochemical coupling of, 314-16
 occurrence of, 314-15
 triphosphopyridine nucleotide and, 315
 photosynthesis and, 247
 reaction of, 309-10
 from wheat germ, 310-11
 Wood-Werkman reaction and, 311
- Manganese**
 availability of, oxidation state and, 8-10
 deficiency of, 9, 13
 exchangeable ions and, 3-4
 malic enzyme and, 312
 in soils, extraction of, 5
 valency of, 9
- Mass flow**
 description of, 120
 in *Arachis*, 122-23
 in *Arenga*, 122
 in palms, 121-22
 translocation and, 124
- Mechanisms**
 of ion absorption, 189-206
 of photoperiodism, 284-87
 of vernalization, 290-91
- Meiosis, desoxyribonucleic acid and, 55**
- Melanin, fruit browning and, 158-59**
- Membrane, of chloroplast, 233**
- Metabolism**
 ion absorption and, 190-91, 203
 trees and, 333-38
- Methemoglobin, Hill reaction and, 251**
- Micronutrients in soils**
 analysis for, 4
 availability of, 1-15
 activity measurements and, 13-14
 aging and, 11-13
- complex formation and, 5-7
 cycle in soil and, 1
 exchangeable ions and, 3-5
 microorganisms and, 14-15
 oxidation and, 8-10
 precipitates and, 7-8
 primary minerals and, 1-3
 recrystallizing and, 11-13
 soluble complexes and, 10-11
 unavailability and, 3-10
 extraction of, 4
- Micronutrient supply, estimation of, 12**
- Microorganisms**
 low temperature plant product preservation and, 165-66
 nutrient availability and, 14-15
- Middle lamella, abscission and, 92**
- Mineral deficiencies, 220**
- Minerals**
 absorption of, 189-206
 in plants, 207-22
 forms of, 213-14
 seasonal trend of, 214-15
 see also Ion absorption; and Plant analysis
- Mitochondria, 25**
 auxins and, 80
 enzymes in, 51-52
 hexokinase and, 25
 Krebs cycle and, 308
 in leaf cells, isolation of, 138
 myokinase and, 25-26
 protein extraction and, 132
 pyruvic acid reactions and, 30
 translocation and, 114
- Mitosis, desoxyribonucleic acid and, 55**
- Models, of ion absorption, 191-200**
- Morphogenesis, auxins and, 67-69**
- Mung bean, mitochondria from, respiration of, 308**
- Myokinase, distribution and action of, 25-26**
- N**
- Nitrogen, virus infections and, 181-82**
- Nitrogen deficiency, chloroplasts and, composition of, 235**
- Nitrogen fractions, in plants, analyses for, 214**
- Nitrogen nutrition, virus diseases and, 177**
- Nuclei**
 desoxyribonucleic acid in, 55
 protein extraction and, 132
- Nucleic acids**
 nuclear division and, 55
 types of, 54
- Nucleoproteins**
 from leaves, 145
 virus and, 171
 virus infection and, 182
- Nutrient availability**
 measurement of, 13-14
 by electrometric method, 13-14
 by silicate membranes, 13
 microorganisms and, 14-15
 soil sterilization, 14
- Nutrient levels**
 in plants, 217-24
 see also Critical nutrient concentration
- Nutrients, critical concentration of, growth and, 217-18**
- Nutrition**
 physiological basis of
 assessing, 207-26
 plant analysis and, 207-26
 of plants, assessment of, 207-26
 virus infection and, 177-78
 see also Plant analysis
- O**
- Ochoa reaction, malic enzyme and, 309-12**
- Off-flavors, in frozen products**
 catalase and, 158
 enzymes and, 157-58
 heat treatment and, 158
 peroxidase and, 158
- Organic acids, auxin action and, 89-91**
- Organic matter, nutrient supply in soil and, 6**
- Osmotic diffusion pump, 194-96**
- Oxaloacetate, decarboxylation of, by cucurbit seed globulins, 311-12**
- Oxaloacetic carboxylase, malic enzyme and, 310**
- Oxidation**
 nutrient availability and, 8-10

- iron and, 10
- manganese and, 9-10
- see also Micronutrients
- Oxidative degradation, of
 - glucose-6-phosphate, 323-26
- Oxygen, ion absorption and, 199-200
- P**
- Palms, bleeding in, 121-22
- Parenchymatous tissue
 - translocation in, 109-10
 - see also Translocation in parenchymatous tissue
- Partial equilibrium, ion
 - transport and, 191-93
- Pears, preharvest drop in, control of, 101
- Pectinic acids, in low temperature preservation, 157
- Pectins
 - auxins and, 91
 - calcium-sparing and, 91
 - cell elongation and, 91
 - June drop and, 91-92
 - methylesterase activity and, 91
- Pentoses
 - origin of, 324
 - phosphorylated compounds and, 37
 - synthesis of, 46
 - aldolase and, 28
- Permeability, ion absorption and, 200
- Peroxidase, off-flavors and, 158
- Phellogen, formation of, in trees, 340
- Phenolic compounds, fruit
 - browning and, 159
- Phenoxyacetic acids, auxin
 - activity of, 62-63, 64, 93
- β -Phenylbutyric acid
 - antiauxins and, 65-66
- Phloem
 - exudation from, 118-19
 - sap concentration of, 119-20
 - sieve tubes in, anatomy of, 117-18
 - translocation in, 117-26
 - accelerated diffusion and, 120
 - mass flow and, 120, 121-23
 - mechanisms of, 120-21
 - simultaneous movement and, 120, 121, 123
 - suction and, 123-24
 - transpiration and, 123-24
 - see also Mass flow; and Sieve tubes
- Phloem necrosis, virus diseases and, 181-82
- Phosphatase, in proteins, 145
- Phosphate metabolism,
 - auxin action and, 89
- Phosphofructokinase, 26
- Phosphoglucumutase, 24-25
- Phosphogluconic acid
 - pentose synthesis and, 46
 - shunt mechanism and, 46
- Phosphoglyceric acid
 - conversion of, 30
 - estimation of, 35-41
- Phosphohexoisomerase, 26
- Phosphopyruvic transphosphorylase, 30
- Phosphorous
 - cytochrome system and, 51-52
 - esterification of Krebs cycle and, 50-52
 - photosynthesis and, 52
- Phosphorous fractions, analyses for, 214
- Phosphorous nutrition, virus diseases and, 177
- Phosphorous turnover, inhibition of, by arsenite, 51
- Phosphorylase, 20, 22-24
 - arsenic and, 23-24
 - auxin and, 77
 - distribution of, 22-23
 - inhibition of, 22
 - in jackbean, 23
 - primer of, 23
 - sucrose and, 24
- Phosphorylated compounds
 - adenosinetriphosphate
 - in Euglena, 39-40
 - identification of, 39
 - isolation of, 38
 - in mung bean, 39
 - in oats, 38
 - and photosynthesis, 52
 - in spinach, 39
 - desoxyribonucleic acid, estimation of, 42-43
 - in disaccharide formation, 47-48
 - estimation of
 - anion exchange resins and, 41-42
 - by barium fractionation, 36-37
 - by chromatographic methods, 40-41
 - early methods in, 36
 - fractionation procedures for, 35-43
 - pentoses and, 37
 - phytic acid and, 38, 39
 - radioautographic methods and, 40-41
 - starch and, 37, 38
 - in glycolysis, 43-46
 - growth and, 52-54
 - adenosinetriphosphate generation and, 53
 - adenosinetriphosphate pool and, 54
 - arsenite inhibition and, 54
 - growth enzyme and, 53-54
 - phytic acid and, 53
 - relation of, 53
 - in Krebs cycle, 50-52
 - metabolism of, 35-55
 - nucleic acids, 54-55
 - nuclear division and, 54-55
 - in photosynthesis, 48-50
 - ribonucleic acid, estimation of, 42-43
 - in starch formation, 46-47
 - see also Glycolysis; and Photosynthesis, specific compounds
- Phosphorylation, auxins and, 81-82
- Photoinduction, flowering and, 266
- Photoperiodic induction,
 - auxin and, 279-80
 - changes in, 278-80
 - levels of, 279
 - in long-day plants, 280
- Photoperiodism, 266-90
 - floral stimulus and, 277-83, 287-90
 - general mechanism of, 284-87
 - definitions in, 284
 - leaves and, 285
 - light in
 - absorption of, 280-82
 - action spectra of, 281-82
 - photoinduction in, 268
 - grafts and, 270-71
 - leaves and, 269
 - photoperiodic types and, 269-70
 - temperature and, 269
 - self-perpetuating effects in, 297
 - vernialization and, 290-96
 - see also Floral stimulus
- Photoreactivation, virus infection and, 175
- Photosynthesis, 229-61
 - of bacteria, 317-18
 - bacteriochlorophyll and, 238
 - C_2 units and, 319-20
 - carboxylases and, 307
 - carotenoids and
 - in brown algae, 238
 - in green algae, 238
 - in purple bacteria, 238
 - chlorophyll and, function

- of, 259-60
 - chloroplasts and, 229-60
 - dicarboxylic acid cycle and, 321
 - energy dissipation and, 249-50
 - formaldehyde and, 323
 - fucosanthol and, 238
 - glycolic acid and, 322-23
 - glycolysis and, 30-31
 - Hill reaction and, 244-54
 - inhibition of, 49, 252-53
 - intermediate hydrogen acceptors and, 248-49
 - malic enzyme and, 314-16
 - phosphorous metabolism and, 48-50
 - phosphorylated coenzymes and, 50
 - phosphorylated compounds and, 49
 - photoinduction and, 278
 - phycoerythrin and, 238
 - pigments in
 - energy absorption by, 237-38
 - functions of, 237-44
 - pyruvate carboxylation and, 317
 - quantum yield and, 250
 - decline of, 241
 - wave lengths and, 241
 - resonance migration and, 241-44
 - ribulose and, 321-22
 - sedoheptulose and, 321-22
 - spirilloxanthin and, 238
 - surface reaction and, 229 of trees, 333-36
 - see also Chloroplasts; Hill reaction; and Malic enzyme
 - Phycobilins, chlorophyll-a and, 241
 - Phycocyanin, in blue-green algae, 231
 - Phycocyanin, photosynthesis and, 238
 - Physiology, of trees, see Trees, physiology of
 - Phytic acid, estimation of, phosphorylated compounds and, 38
 - Pigments
 - in chloroplasts, functions of, 237-44
 - see also Chloroplasts; and Photosynthesis
 - Pineapple
 - auxins and, 88
 - flowering of, 90, 97
 - auxins and, 74-75
 - plant analysis and, 224-25
 - Plant analysis
 - fresh versus dried material in, 208-9
 - history of, 207-8
 - interpretation of
 - critical nutrient concentration and, 217-24
 - deficiencies and, 224
 - response of plants and, 225
 - sampling time and, 224-25
 - nutrition requirements and, 207-26
 - plant part for
 - constituents in, 213-14
 - copper distribution and, 210
 - fertilization and, 210
 - fruits and, 212-13
 - in grape, 211-12
 - in pineapple, 212
 - sampling of, 213
 - seasonal trends and, 214-15
 - time of collection of, 213
 - in tobacco, 210-11
 - in tomato, 210-11
 - in various plants, 212
 - zinc distribution and, 210
 - Purdue test kit and, 208
 - sampling frequency and, 216
 - techniques of, 208-17
 - use of, 225-26
 - Plant products, low temperature preservation of, physiological aspects of, 149-66
 - Plasmolysis, of sieve tubes, 118
 - Polyphenoloxidase, fruit browning and, 158-59
 - Polysaccharides
 - synthesis of, 46-48
 - see also specific compounds
 - Poplar, breeding of, 343
 - Potassium
 - accumulation of
 - by frog's muscle, 190-91
 - in model, 198-98
 - exchangeable ions and, 4
 - source of, in soils, 2
 - Potato sprouting, prevention of, by various compounds, 103
 - Precipitates
 - nutrient availability and, 7-8
 - see also Micronutrients
 - Preharvest drop
 - auxins and, 100-2
 - see also Fruit drop
 - Primary minerals
 - nutrient supply by, 1-3
 - adensine and, 2
 - bytownite and, 2
 - calcium and, 2
 - climate and, 2
 - cobalt and, 2
 - copper and, 2
 - geochemical approach and, 3
 - hypersthene and, 2
 - magnesium and, 2
 - potassium and, 2
 - sand and, 2
 - silicate minerals and, 2
 - weathering and, 2
 - zinc and, 2
 - see also Micronutrients
 - Primary photosynthetic pigment, chlorophyll a, 238-40
 - Proteins
 - in chloroplasts, 135-38, 232-33, 235
 - denaturation by vacuolar sap, 133-44
 - in leaf cytoplasm, 139-47
 - in leaves, 131-47
 - in mitochondria, 138
 - viruses and, 182-84
 - see also Chloroplasts, proteins in; Cytoplasmic proteins; and Leaf proteins
 - Protein synthesis, growth and, auxins and, 78-79
 - Protoplasmic streaming, inhibition of, 51
 - Pseudoamylose, 20
 - Purdue test kit, 208
 - Purple bacteria
 - bacteriochlorophyll in, 231
 - carotenoids in, 231
 - chromatophores in, 231
 - grana in, 231
 - pigments in, 231
 - Pyrophosphate
 - action of, 7
 - as extractant, 7
 - micronutrients and, 10-11
 - nutrient supply in soil and, 6-7
 - soluble complex formation by, 10-11
 - Pyruvate formation, inhibition of, 43
 - Pyruvic acid, reaction paths of, 30
 - Pyruvic carboxylase, 318-19

Q

- Q-enzyme, 19-22
 - action of, 19-20
 - amylopectin and, 19-22
 - crystallization of, 21
 - isophosphorylase and, 21
 - phosphorylase and, 20
 - starch synthesis and, 47
- Quantum yield
 - of Hill reaction, 252
 - of photosynthesis, 241, 250

R

- Radioautograph, of zinc, in plant, 210
- Radioautographic methods, phosphorylated compounds and, 40-41
- Recrystallization nutrient availability and, 11-13
 - see also Micronutrients
- Reproduction, in flowering plants, 285-300
- Respiration
 - auxins and, 79-82
 - growth and, 53-54
 - Krebs cycle and, 307-9
 - in roots, 201-2
 - salt transport in roots and, 115-16
 - of trees, 333-36
 - see also Auxins, respiration and; Glycolysis; and Krebs cycle
- Riboflavin phosphate, estimation of, 40
- Ribonucleic acid, estimation of, 42-43
- Ribulose
 - origin of, 321-22
 - photosynthesis and, 322
- Roots
 - aldolase in, 27
 - antiauxins and, 68-69
 - auxins and, 68-69
 - translocation in, 114-16
 - see also Translocation, in roots

S

- Salts
 - absorption of, 189-206
 - accumulation of, 189-206
 - translocation of, 116-17
 - see also Ion absorption
- Sampling
 - for plant analysis, 213-16
 - time of, plant analysis and, 224-25
- Sand, nutrient supply by, 2-3
- Sap flow
 - in trees, 336-38
 - see also Trees, sap flow in
- Sap of trees, composition of, 337-38
- Secretion
 - ion transport and, 203
 - in roots, 114-15
 - in trees, 338
- Sedoheptulose
 - color test for, 321
 - occurrence of, 321
 - photosynthesis and, 321-22
- Selectivity, ion absorption and, 190

- Self-perpetuating effects
 - in floral initiation, 296-98
 - in photoperiodism, 297
 - in vernalization, 297
- Sex differentiation, in flowers, 300
- Short-day plants
 - floral initiation in, 275-77
 - flowering of, auxins and, 71-72
- Sieve tubes
 - absorption and loss by, 125-26
 - in angiosperms, 117
 - contents of, composition of, 338
 - in Dioscoraceae, bast glomeruli in, 117
 - in gymnosperms, 117
 - life of, 118
 - plasmolysis of, 118
 - sieve plates in, 118
 - transport in, of fluorescein, 124-25
- Silicate minerals, nutrient supply and, 2
- Simultaneous movement, translocation and, 120, 121, 123
- Sodium
 - accumulation of, in model, 196-98
 - exclusion of, by frog's muscle, 190-91
- Soil sterilization, micronutrient supply and, 14, 15
- Soluble complexes
 - nutrient availability and, 10-11
 - see also Micronutrients
- Spirilloxanthin, photosynthesis and, 238
- Starch
 - formation and phosphorylation of, 19-24
 - phosphorylated compounds and, 37-38
 - synthesis of
 - adenosinetriphosphate and, 48
 - amylopectin and, 46-47
 - amylose and, 46-47
 - isophosphorylase and, 47
 - Q-enzyme and, 46-47
 - see also Glycolysis, starch and
- Stationary equilibrium, ion transport and, 193-200
- Strontium, exchangeable ions and, 4
- Succinic acid, photosynthetic formation of, 317-18
- Sucrose, synthesis of, 47-48
 - inhibition of, 48
 - photosynthesis and, 47

- polysaccharide synthesis and, 48
- Sugar, in low temperature preservation, 157
- Sugar beet, control of flowering in, 97
- Sugar cane, plant analysis and, 224-25
- Synthesis
 - of pentoses, 28
 - of starch, 19-24

T

- Tannin, procurement of, 339
- Tannins, fruit browning and, 159
- Temperature
 - photoinduction and, 269
 - virus infection and, 175-77
- Thiamine pyrophosphate, in glycolysis, 44
- Thinning, of trees, 343
- Tobacco leaf proteins
 - isolation of, 143-45
 - properties of, 145-46
- Translocation
 - circulation systems and, 127
 - definition of, 109
 - 2,4-dichlorophenoxyacetic acid, 123
 - direction of
 - growth phase and, 126-27
 - materials utilization and, 126-27
 - of floral stimulus, 282-84
 - of flower-inducing hormone, 123
 - from leaves, 125-26
 - mass flow and, 124
 - in parenchymatous tissue
 - of asparagine, 111-12
 - of caffeine, 111-12
 - of chloride, 112
 - in cotton, 110
 - in Cuscuta, 110
 - in Drosera, 112-13
 - energy for, 114
 - inhibition of, 113-14
 - of lithium, 111
 - mitochondria and, 114
 - in Pelargonium, 110
 - in Statice, 110
 - in Vallisneria, 111-12
 - vital stains and, 110-11
 - in phloem, 117-26
 - in roots
 - anion respiration and, 114
 - cytochrome oxidase and, 114
 - epidermis and, 114-15
 - ion release and, 115
 - neutral red and, 115
 - respiration and, 115-16

- secretion and, 115-16
vacuoles and, 115
- of salts
calcium and, 116-17
circulation and, 116
tracers and, 116
in sieve tubes, fluorescein
and, 124-25
in trees, 336-38
of virus, 123
see also Floral stimulus,
translocation of;
Phloem; Sieve tubes;
and Trees, sap flow
in
- Transpiration
translocation and, 123-24
of trees, 333-36
- Transport
of ions
in partial equilibrium,
191-93
in stationary equilibri-
um, 193-200
of organic compounds,
109-27
see also Translocation
- Tree physiology, definition
of, 33
- Tree rings
in barks, 340
effect of drought on, 340-
41
formation of, 339
synchronization of, 340-41
- Trees
Absprunge of, 340
assimilate flow in
daily march of, 335, 337
velocity of, 337
breeding of
heterosis and, 343
reproduction and, 342
results of, 342-43
seed plantations and,
341-42
thinning and, 343
gas exchange of
determination of, 333,
335-36
table of, 334
growth of
cambial activity and,
339, 341
early wood and, 339
late wood and, 339
mathematical expression
of, 338
periderm in, 340
seasonal variation in,
339
growth and development
of, 338-41
metabolism of, 333-38
physiology of, 333-43
reproduction and, 341-43
respiration of, bark and,
336
rings in
drought and, 340-41
various localities and,
340
sap of, composition of,
337-38
sap flow in
annual variations of,
337
daily march of, 337
nature of, 336-37
secretion in, 338
sieve tube contents, com-
position of, 338
transpiration of
annual march of, 336
bark and, 336
drought and, 334-35
water content of, seasonal
changes, 337
- 2,3,5-Triodobenzolic acid
antiauxins and, 67-68
flowering and, 73
- Triosephosphate dehydrogen-
ase, 28-30
distribution of, 28-29
in glycolysis, 45-46
inactivity of, 29-30
inhibitors and, 28, 45-46
photosynthesis and, 49
- Triphosphopyridine nucleo-
tide, photosynthesis
and, 50, 247-49
- Tryptophan, indoleacetic
acid synthesis and,
61
- U
- Ultraviolet irradiation, virus
infection and, 175
- V
- Vacuoles
ion transport and, 115
protein extraction and,
133-34
- Vernalin, floral stimulus and,
293-94
- Vernalization
chemical changes in,
291-92
floral stimulus in, 292-94
mechanism of, 290-91
photoperiodism and, 290-
96
self-perpetuating effects
in, 297
- Virus
animal versus plant, 171
infection by, host metabo-
lism and, 178-80
infectivity of, 172-73
susceptibility to, definition
of, 172
synthesis of, 183
translocation of, 123
see also Virus infection
- Virus diseases
nature of, 171
physiology of, 171-85
see also Virus
- Viruses
auxins and, 92-93
cytoplasmic proteins and,
146-47
determination of, 133-34
isolation of, 183-84
- Virus infection
age of host and, 178
effect of, 180-85
carbohydrate/nitrogen
ratio and, 181-82
on host enzymes, 180-81
illumination of host and
photoreactivation and,
175
susceptibility to, 174-75
symptoms and, 174
ultraviolet radiation and,
175
- inhibition of
by antibiotics, 179
by other infections, 180
by ribonuclease, 178-79
by various substances,
179-80
- leaf-hopper growth and,
184
- leaf proteins and, 182-83
methods of study, 173
nucleoproteins and, 182
nutrition of host and
nitrogen in, 177-78
phosphorous in, 177
phloem necrosis and, 181
seasonal effects on host
and, 173-74
starch metabolism and,
181-82
symptoms of
auxins and, 184-85
bases for, 184
temperature and
heat therapy and, 176
susceptibility to, 176-77
symptoms of, 175-76
- Virus multiplication, nature
of, 185
- Vital stains, translocation
and, 110-11
- W
- Water uptake
auxins and, 82-83
2,4-dinitrophenol and, 83
respiration and, 82
see also Auxins, water up-
take and
- Weed control, 87
2,4-dichlorophenoxyacetic

SUBJECT INDEX

369

acid in
action of, 95
activity of, 96
fate in soil of, 96-97
preference for, 96
Wood
tree rings in, 339-41
water content of, 337

Wood-Werkman reaction, 311

X

Xanthium, flowering of,
control of, 72-73
X-hypothesis, nutrient sup-
ply in soil and, 6

Z

Zinc
aldolase and, 27
distribution, in plant, 210
exchangeable ions, 4
phosphofructokinase and, 26
source of, in sand, 2-3